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# Seasonal Variation in Diet of a Marginal Population of the Hispid Cotton Rat, Sigmodon hispidus

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#### **ABSTRACT**

Cotton rats live in oldfields, habitats with a variety of mostly herbaceous plants. Based on other studies, the hispid cotton rat, Sigmodon hispidus, eats many kinds of herbaceous plants but grasses predominate. In contrast, our population of cotton rats ate many monocots but mostly they were not grasses. Our study sought to determine the diet of the cotton rat in eastern Virginia, near the northern limit of distribution on the Atlantic Coast. Fecal samples, collected each month during an on-going capture-mark-release demographic study of the rodent community, were analyzed using a standard method. A greater variety of foods (including insects) was eaten in the summer and autumn than in winter and spring. In winter, when much herbaceous vegetation is standing dead, cotton rats supplemented their diets with pine bark. Cotton rats ate significantly greater proportions of monocots in winter and spring, an apparent response to the need for more calories to compensate for greater heat loss. In summer and autumn, cotton rats enhanced their diets with significantly greater proportions of the more nutritious but harder to digest dicots. Reproductive females ate significantly more dicots and less monocots than males and non-reproductive females, whose diets were similar. Key words: cotton rat, diet, fecal analysis, pine bark, plant availability, seasonal variation, Sigmodon hispidus

#### INTRODUCTION

Studies of diet, at their simplest, reveal whether mammals are herbivores, carnivores, omnivores, or consume specialized foods such as ants. When evaluated throughout an annual cycle, dietary studies also can indicate the role of that species in its community and how diet may change with the seasons, with the changing energy requirements of reproduction, impending migration or hibernation (Parker and Bernard, 2006). Diet can be assessed by direct observation, feasible primarily for large diurnal mammals, or by analysis of the contents of stomach or feces (Colgan et al., 1997).

Previous studies of the diet of the hispid cotton rat (Sigmodon hispidus), conducted using fecal analysis, have shown that this rodent is mostly herbivorous but occasionally also consumes insects. Examined in coastal Texas throughout an annual cycle (Kincaid and Cameron, 1985) and in western Kansas in the summer (Fleharty and Olson, 1969), the latter representing a marginal population, the diet of cotton rats varies greatly due to the large variety of plant species in their habitats and to their differences in nutrient

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content, secondary plant compounds, palatability and digestibility (Randolph et al., 1991). These features undoubtedly change during the year, with foods likely of poorest quality and lowest amount during the winter months. Diet may reflect the availability of plant species, but often it does not (Randolph et al., 1991).

Cotton rats consume mostly monocots, especially grasses, but also eat dicots (Kincaid and Cameron, 1985). Laboratory studies show that monocots are digested more rapidly and thus provide calories easily, whereas dicots are more nutritious but take longer to digest (Randolph et al., 1991). These factors can contribute to the differential selection of food plants by cotton rats of different reproductive states living in different months of the year. Knowledge of the diet of the cotton rat near the northern limit of distribution in the mid-Atlantic region will permit comparisons with more central and other peripheral populations. Using fecal samples collected throughout the year, our study sought evidence of seasonal variation in food selection, including information on the dominant plant species consumed in different seasons. We also compare differences in food selection among males, non-reproductive females, and reproductive females.

#### MATERIALS AND METHODS

#### Study Area

The study site was an 11-ha tract owned by The Nature Conservancy in southern Chesapeake, Virginia. The flora consisted of several monocots, including Juncus effusus and J. tenuis (soft rushes), Schizachyrium scoparium (little bluestem), Allium vineale (field garlic), Carex spp. (sedges), Panicum spp. (switchgrass), Scirpus cyperinus (a sedge, wool-grass), and Microstegium vimineum (Nepalese browntop grass). Also present was a variety of dicots: Symphyotrichum pilosum (awl-aster), Solidago spp. (goldenrods), Solanum carolinense (horse nettle), Campsis radicans (trumpet creeper), Lonicera japonica (Japanese honeysuckle), Apocynum cannabinum (hemp-dogbane), Eupatorium capillifolium (small dog-fennel), Rubus allegheniensis (blackberry), and Ambrosia artemisiifolia (common ragweed). The site was being rapidly invaded by loblolly pine (Pinus taeda) trees that gradually decreased the amount of herbaceous ground cover, originally dominant. In the wettest areas, soft rushes, wool grass and asters were the dominant plants. In addition to cotton rats, other small mammals in the community, in order of their decreasing abundance, were: Microtus pennsylvanicus (meadow vole), Reithrodontomys humulis (eastern harvest mouse), Oryzomys palustris (marsh rice rat), Mus musculus (house mouse), and Blarina carolinensis (southern short-tailed shrew).

#### Field Methods

A 1-ha grid, consisting of eight rows of traps with eight stations at 12.5 m intervals along each row, was placed in a grassy section of the field in December 2002. Every coordinate had two Fitch live traps (Rose, 1994) that were baited with birdseed and filled with polyfill as insulation in the winter months. The traps were set one afternoon and then checked for three consecutive mornings each month. Half sheets of copy paper, placed under the mesh when setting traps, collected feces on the first morning of trapping. The feces from each animal were folded in the paper, which was labeled with information on sex, weight, date, and tag number, and then stored in a freezer. Fecal samples from 53 cotton rats, with 10-15 samples per season, were analyzed. The seasons were defined as autumn (September-November), winter (December-February),

spring (March-May) and summer (June-August).

Because the cotton rats analyzed in our study were part of an ongoing capture-mark-release study (which followed the guidelines of the American Society Mammalogists for the use of wild mammals in research: Gannon et al., 2007), analysis of diet using only fecal samples was possible. For each animal, we also recorded information on its reproductive status. For males, the testes were recorded as scrotal (reproductive) or abdominal (not reproductive). For females, reproductive information included if pregnant, whether vagina was perforate or not, nipple size (small, medium, and large), and the condition of pubic symphysis (closed, slightly open, or open). Together these features permit an assessment of reproductive condition (McCravy and Rose, 1992). Lab Analysis of Plant Parts and Fecal Samples

Each month during spring and summer of 2006, plants were collected and identified in the field; identifications were verified by a plant expert (Dr. Rebecca Bray and Jay Bolin) in the department. Later, parts of the plants were used to make reference slides for each species, using the procedures of Davitt and Nelson (1980). Pieces of a plant were placed in a lactophenol-blue stain for 7 d and then macerated in a Waring blender for 3-5 min. The smallest plant particles were removed from the surface of the water using a tiny wire loop, placed on a microscope slide (two slides per reference plant), and then dried on a hot plate. Once the slide was dry, a mounting medium and coverslip were added to create a semi-permanent reference slide for each plant species (Davitt and Nelson, 1980).

Five to six fecal pellets per sample were also subjected to the lactophenol-blue stain, then ground with mortar and pestle, the smallest pieces again were looped from the surface of the water, mounted on a slide and dried. Two slides per sample were prepared as before. Twenty-five random microscope fields were examined per slide (50 fields/sample). If 100X magnification was inadequate for identification, higher magnification was used.

The reference slides were used to identify the plant fragments in each fecal sample. Unique micro-anatomical features, such as epidermal hairs, size/shape of cells, trichomes, and stomates were used to identify the plant pieces (Sparks and Malcheck, 1968). For each fecal sample slide all particles of each plant species (plus unidentifiable pieces) were counted in each field of view. After these values were summed in all 50 fields, these totals were divided by the total number of fragments of all the species (and unidentifiable parts) per sample and then multiplied by 100 to give percent frequencies of species consumed by the cotton rat (Holecheck and Gross, 1982). This method resulted in a list of the plant species in each fecal sample and their relative proportions, expressed as percent frequencies. Five categories were used: unknown (unidentifiable fragments caused by excessive digestion or distortion), monocots, dicots, pine bark and insects. This information was then merged with information on the sex, reproductive status of the animal and season to give a picture of the changing diet of the cotton rat throughout the year.

Fecal samples used in the analysis were collected from September 2004 through August 2005. Plant abundances were not determined due to the rapid succession in the oldfield between the time the fecal samples were collected and the time that plant samples were collected (spring and summer 2006).

#### Data analysis

All statistical tests for the diet analysis were performed using SPSS for Windows

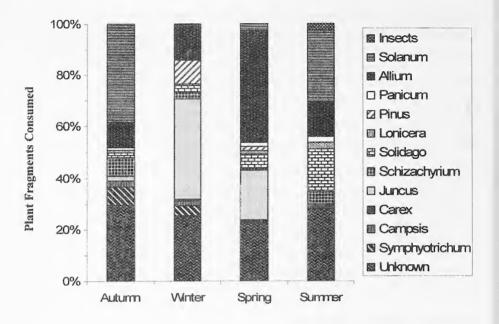


FIGURE 1. Percentages of insects and plant species identified in fecal samples in each of four seasons (n = 53: 10-15 per season).

2005, version 14.0, with significance levels of P < 0.05 (SPSS Inc., Chicago, Illinois). The percent frequencies of diet were tested for normality and homogeneity of variances using Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. The unidentifiable components, monocots, and dicots for the seasons were normally distributed but due to the multiple zeros recorded for pine bark and insects, those variables were not normally distributed. After multiple transformation attempts, the tests of normality still could not be met satisfactorily for pine bark and insects. Therefore, percent frequencies were used in a general linear model (GLM) multivariate analysis (MANOVA) to compare the diet composition between season and sex in relation to major food types. If there was a significant effect from either the season or sex on a major food type in the GLM MANOVA, a one-way ANOVA Ryan-Einot-Gabriel-Welsch-F test (REGWF) was performed to test the significance of each major food type individually against each season or sex.

#### RESULTS

## Effects of Season on Percent Food Consumption

The percent of unknown plant fragments was relatively constant across the four seasons, ranging from 22-29% (Fig. 1). The following were the primary foods consumed: monocots (*Juncus*, *Schizachyrium*, *Allium*, *Carex*, and *Panicum*), dicots (*Symphotrichum*, *Solidago*, *Solanum*, *Campsis*, and *Lonicera*), pine bark, and insects; these four groups were used in analysis.

The most important plant species varied among seasons (Fig. 1). In autumn, Solanum represented 39% of the diet, with Symphyotrichum, Schizachyrium, and Allium

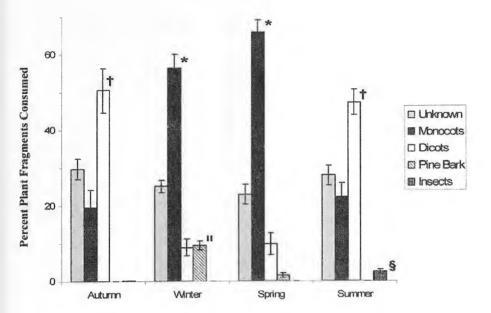


FIGURE 2. Percentages (mean  $\pm 1$  SE) of plant fragments, and insects, separated into food categories eaten for each season. The following symbols are used: † proportions of dicots consumed were significantly greater in summer and autumn than winter and spring (P < 0.01), \* proportions of monocots consumed were significantly greater in winter and spring than summer and autumn (P < 0.01), \* proportions of pine bark consumed was significantly greater in winter than spring, and § proportions of insects consumed was significantly greater in summer than autumn (P < 0.01).

as minor (7-10%) components. During winter, the diet primarily consisted of *Juncus* (39%), *Allium* (14%), and pine bark (9%). In spring, *Juncus* and *Allium* were dominant again, but their proportions were reversed: 44% for *Allium* and 20% for *Juncus*. Finally, in summer, a greater variety of plants was eaten, with *Solanum*, *Solidago*, and *Allium* consumed the most at 28%, 17%, and 14%, respectively. Despite its availability, *Juncus* was not eaten in summer, perhaps due to the variety of other plant species available then.

In winter and spring, monocots were 56% and 66% of the identifiable diet and dicots were only 9% and 10%, respectively (Fig. 2). The greater use of monocots in winter and spring versus summer and autumn was significant based on the post-hoc REGWF test (P < 0.01). A significantly greater proportion of pine bark was eaten in the winter than in spring (P < 0.01). In summer and autumn, dicots made up 47% and 50%, respectively, seasons when monocots constituted less than 25% of the diet (Fig. 2). This preference for dicots in the summer and autumn was significantly greater (P < 0.01) than in the winter and spring. Significantly (P < 0.01) more insects were eaten in summer (2.5%) than in autumn (0.1%); both are seasons of greatest availability.

## Effects of Sex on Percent Food Consumption

Over the year, males (n = 18) ate 43% monocots and 30% dicots and non-

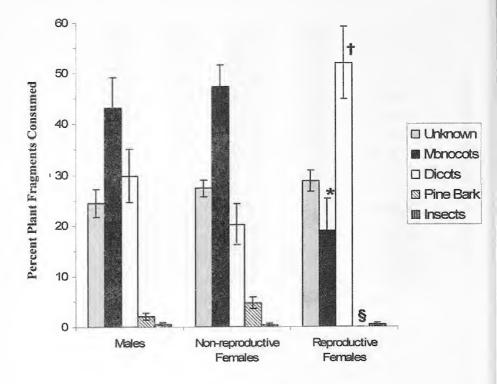


FIGURE 3. Percentages (mean  $\pm 1$  SE) of plant fragments, and insects, separated into food categories for males (M), non-reproductive females (NRF) and reproductive females (RF), with n=18, n=27, and n=8, respectively). The following symbols are used: \*proportions of monocots consumed by RF was significantly less than M and NRF (P < 0.05), † proportions of dicots consumed by RF was significantly greater than M and NRF (P < 0.05) and § proportions of pine bark consumed by RF was significantly less than M and NRF (P < 0.05).

reproductive (n = 27) females consumed similar proportions, at 47% and 20%, respectively (Fig. 3). However, reproductive females (n = 8) consumed 19% monocots and 52% dicots. Post-hoc tests showed that reproductive females ate significantly (P < 0.05) less monocots and more dicots than males and non-reproductive females. Males and non-reproductive females consumed significantly (P < 0.05) more pine bark (2% and 4.7%, respectively) than reproductive females, which ate none. The amount of insects consumed, never greater than 1%, did not differ among the three groups. Males and non-reproductive females consumed relatively similar proportions of monocots, dicots, pine bark, and insects (P > 0.10).

#### DISCUSSION

Cotton rats are short-lived animals that grow rapidly with an average lifespan varying from 2-3 months depending on location (Cameron and McClure, 1988). Due to the short lifespan, cotton rats cannot substantially benefit from having selected or avoided certain plants earlier in the year (Randolph et al., 1991). At our site, they consumed varying proportions of 10 common herbaceous plants, a few insects when

available and at one time of the year, pine bark. During the year, different monocots and dicots were eaten, presumably depending upon their availability, palatability and nutritional values.

Proportions of monocots, dicots, pine bark and insects eaten varied with the season. Monocots typically were eaten more in the winter and spring, when they represented greater than 50% of the identifiable diet (Fig. 2). Especially in winter, relatively little living green herbaceous vegetation except Allium and Juncus (both monocots) was available (Fig. 1). During winter and spring, when the food selection likely was limited, cotton rats consumed pine bark to supplement their diets (Fig. 1). Even though pine bark contains noxious resins and tannins, cotton rats at our site consumed it in relatively large amounts, especially in late winter (Fig. 2). Cotton rats ate pine bark at a time when nutrient-rich sap is rising from the roots and when food quality probably is lowest in overwintering herbaceous plants. We assume they are improving their diets by eating bark. Other rodents known to eat the bark of conifers, not necessarily at one time of the year, include the Abert's squirrel (Sciurus aberti), which eats phloem and cambium of the bark of the ponderosa pine in western North America as the primary winter food (Snyder, 1992) and in the United Kingdom the introduced gray squirrel (Sciurus carolinensis) strips and eats pine bark, mostly in May-July, when bark is stripped most easily (Dagnall et al., 1998).

In summer and autumn the reverse pattern was observed, with cotton rats eating significantly more dicots than monocots; dicots comprised ca. 50% of their diet then (Fig. 2). The most common dicots eaten during the summer and autumn were *Solanum* (28 and 39%, respectively), and *Solidago* (17 and 2%) (Fig. 1). Although pine bark was not found in fecal samples from summer and autumn, insects were (Fig. 2). Kincaid and Cameron (1982) believed insect consumption, highest in the summer, was by incidental ingestion, but our results suggest active insect consumption. Of the 25 cotton rats sampled in summer and autumn, 10 (40%) had eaten insects (Fig 2). Active eating of insects is also supported by high infestation rates (25-73% per month, R. Rose, unpublished) in local cotton rat populations of the stomach worm, *Mastophorus muris*, for which insects such as crickets, grasshoppers, and others are the intermediate hosts.

Many studies (e.g., Fleharty and Olson, 1969; Kincaid and Cameron, 1985; Randolph and Cameron, 2001) have reported that cotton rats primarily eat grasses. In Kincaid and Cameron (1985), grasses were highly dominant, comprising the majority of foods consumed in most seasons and 74% of the diet across the year. After learning that foods of higher nutritional content, such as dicot leaves, were typically eaten in summer, Randolph and Cameron (2001) concluded that cotton rats had to compensate for the longer handling time of dicots by trying to decrease the search time. Fleharty and Olson (1969), in a summer-only study in Kansas, also found that dicots were consumed more than grasses then. The food consumed in the highest percentage volume was *Triticum aestivum* (wheat, a grass) at 20%, but in aggregate, forbs comprised 48% of summer diet (Fleharty and Olson, 1969). In our study, dicots were eaten in large amounts in summer and autumn, whereas monocots (including grasses) were consumed more in the winter and spring.

Kincaid and Cameron (1985) found that grasses were consumed most frequently during autumn and winter in the Texas coastal prairie, whereas dicots were consumed more in spring and summer. This is a seasonal shift in pattern compared to our results,

and is probably due to the geographic variation in flowering phenology of food plants. Further, our dominant and important plant species (*Juncus, Solanum, Allium, Solidago*) were absent in the Kansas study and, except for *Solidago*, in the Texas study too. All three studies showed dicots were consumed more than monocots in the summer and the opposite pattern in the winter. However, we never found grasses to be dominant plants in the diet. Of the grasses, *Schizachyrium* was consumed the most in autumn at 7% and *Panicum* the most in summer at 2%. Consequently, in our study grasses comprised only 5% of the average cotton rat diet, which is drastically different from the 74% reported by Kincaid and Cameron (1985). Besides these differences, pine bark, previously unreported as food of cotton rats, was an important component in the diet for a short period at the end of winter. Thus, our study shows the dietary flexibility required of small mammals that can continue to expand their distribution, as *Sigmodon hispidus* has done for the past century (Cameron and McClure, 1988).

Among the factors that determine which species of plants a cotton rat consumes are stage of growth, palatability (Fleharty and Olson, 1969), and search, handling and digestion times (Randolph and Cameron, 2001). Dicots are high in protein but require longer handling times than monocots, whereas monocots have shorter handling times but are not as nutrient rich as dicots (Randolph et al., 1991). Randolph and Cameron (2001) found that differences in both search and handling times played a role in diet selection among seasons. This likely was the case in our study because cotton rats on our grid could find monocots easily during all seasons, with *Juncus* and *Allium* being widely distributed and common on the grid and among the few green foods available in winter and early spring. Accessible foods in winter and spring were mainly monocots, which are easy to break down and convert into energy rapidly. In summer, when cotton rats do not lose as much energy to heat loss as in other seasons, they can afford to eat foods that take longer to find and digest, such as dicots, because they do not need to catabolize energy so rapidly from food then, except perhaps for lactating females.

When examining food consumed over the entire year, a few differences were noted between the sexes (Fig. 3). Males and non-reproductive females consumed 2% and 4.7% of pine bark, respectively. (Pine bark was being consumed in late winter and early spring before females were actively breeding.) Randolph et al. (1991) reported that seasonal diets of males and non-reproductive females were similar except in winter. Our sample sizes were too small to examine differences between sexes among seasons, but males and non-reproductive females had similar diets across the year. Furthermore, reproductive females ate more dicots (52%) than either non-reproductive females or males (20 and 30%, respectively; Fig.3). Also reported by Randolph et al. (1991), this behavior suggests that reproductive females take an active role in meeting the nutritional requirements of pregnancy and lactation.

Randolph et al. (1995) reported that cotton rats fed lab chow had enough energy to meet reproductive requirements, but females in the field had levels of protein and phosphorus too low to meet the demands required for reproduction in both autumn and winter. Females need more energy during lactation than in pregnancy and are constantly balancing between energy lost through heat dissipation and reproductive costs. Due to the high nutritional demands of reproduction, it is important that females eat foods high in protein, which may be the reason reproductive females in our study ate relatively more dicots than non-reproductive females. In an experimental field

study in Texas during which high-protein foods were introduced into some natural habitats, cotton rats chose habitats with high quality foods even when covering vegetation was low, indicating an ability to evaluate food quality (Eshelman and Cameron, 1996). Males and non-reproductive females can afford to be less choosy about the foods they consume because they do not need as much energy (Randolph and Cameron, 2001), which may explain their similar diets.

In conclusion, cotton rats in eastern Virginia exhibited significant seasonal variation in food selection, including the unexpected consumption of pine bark and much lower proportions of grasses eaten than reported for other geographic populations. Significant diet differences were observed between reproductive females and either males or non-reproductive females. The catholic diet of the hispid cotton rat probably has contributed to its range expansion in the last 100 years, including into southeastern Virginia, where its path northward currently is blocked by the Chesapeake Bay and its associated large rivers.

#### **ACKNOWLEDGMENTS**

We thank The Nature Conservancy for the use of their land as a field site, graduate students H. Green, M. Wicks, K. Proctor, T. Egerton, and Rebecca Bray for field assistance, and Dr. R. Bray and J. Bolin for verifying plant identifications.

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# Graminicolous Fungi of Virginia: Fungi in Collections 2004-2007

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#### **ABSTRACT**

Fungus-grass associations recognized in Virginia from 2004 to 2007 are recorded. Many associations are new to the United States (U), eastern United States (EU), and Virginia (V); other associations extend the known distribution of those previously discovered. These reports contribute further to knowledge of the mycoflora of Virginia.

#### INTRODUCTION

Fungi identified on members of the plant family Poaceae since 2003 (Roane 2004) are catalogued here. The objectives were to collect grasses growing in diverse environments and determine which fungi were present or emerged from them. There was no attempt to demonstrate parasitism but merely to establish their presence. Thus, the catalogue becomes a contribution to the natural history of Virginia. As before, any fungus or grass-fungus association not listed by Farr et al. (1989) nor in the web-site (Farr and Rossman date unknown) is considered a new United States record (NR, U). Those not listed east of the Mississippi River are designated NR, EU; those new for Virginia are designated NR, V.

In previous publications, brief descriptions of collection sites were provided; in this publication, elevation and geographic coordinates of collection sites were also provided if feasible by using a hand-held Etrex® GPS receiver (Mfd. by Garmin Ltd., Olathe, Kan.). The instrument provided repeatable coordinate readings but elevations sometimes varied plus or minus 100'.

An acquisition number is provided for each collection. Each is preceded by R, Roane, the last two digits of the year followed by the acquisition number for the year (ex, R07-1). If the specimen originated from the Plant Disease Clinic, i.e., specimens sent in for identification, the Clinic acquisition number is also included (ex, Pl.Cl. 07-1). Collections of ample material will be deposited in the herbarium of the Systematic Mycology and Microbiology Laboratory, ARS, USDA, Beltsville, Md.

Several literature citations appear repeatedly; three that appear in the text are as follows: Ellis and Ellis, E and E; Roane and Roane, R and R; Shoemaker and Babcock, S and B. In text, N.S.R.R. = Norfolk Southern Railroad.

There have been several changes in the binomials of grasses since the publication of the catalogue by Farr et al. (1989). It is simpler to use the binomials listed by them

and recognize in synonymy the names appearing in the Flora of North America (1993), especially since the book by the former is more readily available to plant pathologists than the latter.

#### 2004-2007 COLLECTIONS

Agropyron repens (L.) Beauv., Syn., Elytrigia repens (L.) Nevski, Elymus repens (L) Gould, quackgrass

Ascomycotina:

*Phaeosphaeria luctuosa* (Niessl in Sacc.) Otani and Mikawa - Collected on White Top Mt., Grayson Co. below the roadside spring, June 27, 2004, El. 5019', N38° 38.058', W81° 36.014', R04-20. Ascospores measured 22-27 X 5 $\mu$ m; 5-septate, 3<sup>rd</sup> cell enlarged, apparently cause of leaf lesions. Illustrated by S and B (1989 pp. 1570-1571) and E and E (1985 pl. 171). NR, U.

Phaeosphaeria nigrans (Rob. ex Desm.) L. Holm - Collected at Walton, Montgomery Co., near Rt. 663 and N.S.R.R. crossing, July 20, 2004, El. 1740' N37° 9.173' W80° 31.01', R04-25. Ascospores 20-28 X 4-5μm, 5-septate, 2<sup>nd</sup> cell enlarged; on spikelets. Illustrated by S and B 1989, p. 1546 and E and E (1985 pl. 172). NR, U.

*Phaeosphaeria tritici* (Garov.) Hedj. – Site and dates of R04-25 above. Ascospores 15-17 X 3-4μm, 3-septate, 2<sup>nd</sup> cell enlarged. On blades. Illustrated by S and B (1989) p. 1528, R04-25. NR, U.

Basidiomycotina - Uredinales:

Puccinia recondita Rob. ex Desm., II, III – Collected at Walton, R04-25 (details above), and Burkes Garden, West end, Tazewell Co. along Rt. 625, July 10, 2005, El. 3161', N37° 05.730', W81° 18.528' R05-13, along Rt. 666, and at east end El. 3197', N37° 14.460', W80° 24.525', R05-14. These collections merely extend the known range in Virginia. Note: P. recondita was originally described as P. triticina Erikss., later as P. rubigo-vera by Winter in Rabenh., still later as P. recondita and recently back to P. triticina (Szabo et al. 2004). Since P. recondita was used in previous publications in this series, its use will be continued (R and R 1994, 1996, 1997; Roane 2004).

Deuteromycotina – Hyphomycetes:

*Rhynchosporium secalis* (Oud.) J. J. Davis – Collected at both Burkes Garden sites above, causing scald, R05-13-14, NR, V.

Deuteromycotina – Coelomyetes:

Phaeoseptoria festucae R. Sprague – Collected on White Top Mt., Grayson Co. below roadside spring, June 27, 2004, as described for R04-20 above (*P. luctuosa*). *P. festucae* var. *Muhlenbergia* is listed on *A. repens* in Wisconsin (Farr et al. 1989). However, this collection while fitting the concept of *P. festucae* in Sprague's key (Sprague 1950) does not clearly fit into a described variety. R04-20, NR, EU.

Stagonospora maculata Castellani and Germano – Collected at both Burkes Garden sites above, R05-13-14. Spores 25-34 X 3.0-3.5 $\mu$ m, slightly constricted at the 3-5 septa, mostly 4 septa. NR, U.

Stagonospora nodorum (Berk.) Cast. and Germ. – Mixed with S. maculata on foliage of Burkes Garden (R05-13) collection. Spores measured 17-21 X 2.5-3.0μm, with 3 septa, not constricted at septa. Reported only once before on A. repens in Giles Co. (Roane 2004).

#### Agrostis spp., bentgrass

Collections were made from only two species of *Agrostis*; they are cited by number in the entries below. *Agrostis gigantea* encompasses several species in grass literature but is used here as in the "Atlas of Virginia Flora. III" (Harvill et al. 1992).

- 1. Agrostis gigantea Roth, Syn., A. alba, A. stolonifera, bentgrass, redtop.
- 2. Agrostis perennans (Walter) Tuckerman, autumn bentgrass.

#### Ascomycotina:

Phaeosphaeria nigrans (Rob. ex Desm.) L. Holm – Collected on **2** in parking lot, Havens Wildlife Management Area. Rt. 622 = Bradshaw Rd., Roanoke Co., July 18, 2004, El. 1610', N37° 19.840', W80° 9.283', R04-23. Ascospores were 24-26 X 4-5 μm, 5-septate, 2<sup>nd</sup> cell enlarged, associated with spots and blade wilt. Previously collected in this association in Pulaski Co. (R and R 1996).

#### Basidiomycotina:

*Puccinia coronata* Cda. – Crown rust, collected on **1** in west end Burkes Garden, Tazewell Co., Aug. 29, 2004. El. 3090', N37° 6.614', W81° 21.516', R04-40. This collection expands the known range of this association in Virginia (Roane 2004; R and R 1996).

*Puccinia recondita* Rob. ex Desm., leaf rust – Collected on **2** at Havens W. M. Area (site R04-23 as above), July 25, 2004, R04-28, and at Pandapas Pond, Montgomery Co., Sept. 4, 2005, R05-36, El. 2106', N37° 16.926', W80° 27.951'. These collections extend the known range of this association in Virginia.

## Deuteromycotina - Coelomycetes:

*Ascochyta sorghi* Sacc. – Collected on **2** at the Havens W. M. Area, Roanoke Co., site above July 25, 2004, R04-28. NR, U.

Colletotrichum graminicola (Ces.) G. W. Wils. - Was collected on 1 in the west end of Burkes Garden, Tazewell Co. (El. 3090'), Aug. 29, 2004, R04-40, and on 2 at the Havens W. M. Area July 25, 2004, R04-28. These associations were reported by R and R (1996); they extend their known range.

*Dilophospora alopecuri* (Fr.:Fr.) Fr. – Collected at Butt Mt. Lookout Area, Giles Co. on 1, Aug. 17, 2004. El. 4186', N37° 22.189', W80° 37.199'. It was found on plants with symptoms of bentgrass seed gall nematode [*Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936] infection (Eisenback and Roane 2006). NR, U.

Septoria secalis Prill. and Delacr. – Collected on **2** on Gap Mt. along Forest Service Rd. ¼ mi. W. from highway U.S. 460, Montgomery Co., Aug. 14, 2005, El. 2377', N37° 16.743', W80° 29.022', R05-33. Although collected from three other locations, this is the first from Montgomery Co.

Andropogon spp., beardgrass

- 1. Andropogon gerardii Vitman, big bluestem.
- **2.** *A. scoparius* Michx. = *Schizachyrium scoparium* (Michx.) Nash, little bluestem.

#### Ascomycotina:

*Mycosphaerella recutita* (Fr.:Fr.) Johans. – Collected on **2** at Pembroke quarry on Rt. 623, Giles Co., Sept. 2, 2007, R07-19, El. 1655', N37° 18.755', W80° 39.222'. Fungus fits description by Dennis (1978). Ascus averaged 32 X 10μm; ascospores 9-14 X 3-4μm. On leaves, especially in necrotic tips. NR, U.

#### Basidiomycotina:

Puccinia andropogonis Schwein. III. – Collected on 2 at N. side of N.S. R.R. off Rt. 660, 0.2 mi W. of underpass, Montgomery Co., Sept. 8, 2005, R05-41a, El. 1845', N37° 09.548', W80° 28.310'. NR, V.

#### Deuteromycotina – Hyphomycetes:

Curvularia brachyspora Boedijn – Collected on 1 at a lot, SW corner Giles and Hearthstone Rds., Blacksburg, Montgomery Co., Oct. 9, 2006, R06-28, El. 2123', N37° 14.288', W80° 24.983'. Conidia are 3-septate, end cells lighter colored than two inner cells, 22-29 X 8-9µm, as described and illustrated by Ellis (1971). This fungus is described as being tropical by Ellis (1971) and Farr et al. (1989). NR, U.

Nigrospora sphaerica (Sacc.) E. Mason – Collected on 1 and 2 at site of *Puccinia andropogonis* above, Sept. 8, 2005, R05-41a, R05-42. It was collected on 1 at site of *C. brachyspora* above, Oct. 9, 2006, R06-28. Fungus is described as a weak parasite and common saprophyte. On 2, NR, U.

## Deuteromycotina – Coelomycetes:

Collectrichum caudatum (Sacc.) Peck — Collected on 1 at site of *Puccinia andropogonis* above, Sept. 8, 2005, R05-42, and on 2 at the site of *M. recutita* above, Sept. 2, 2007, R07-19. Some authors assign this fungus to *Ellisiella caudata* Sacc. because the spores taper into an apical appendage. See Sutton (1980) for discussion of nomenclature. The fungus has been found previously on several other grasses (R and R 1996 pp. 202, 207; 1997 pp. 18, 38). On 1, NR, U; on 2, NR, V.

*Phaeoseptoria festucae* var. *andropogonis* R. Sprague – Collected on 1 at the site of *Puccinia andropogonis* above, Sept. 8, 2005, R05-42. Apparently causing leaf spots. The fungus was described in a publication by Greene (1949); there are no other records of this fungus. Spores measured 105-190 X 5-7 $\mu$ m, with 6-10 septa, usually constricted at the septa. NR, EU.

Stagonospora arenaria (Sacc.) Sacc.—Collected on **2** at the *Puccinia andropogonis* site above, R05-41a. This is a common graminicolous pathogen causing a purple brown leaf blotch. Farr et al. (1989) list no Deuteromycotinae in Virginia. NR, V.

Stagonospora simplicior Sacc. and Briard – Collected on **2** along with *S. arenaria* above, R05-41a. They are very different; *S. arenaria* spores are 1-4, usually 3-4-septate, measure 25-60 X 3-5  $\mu$ m; those of *S. simplicior* are 3-septate, 24-44 X 9-11  $\mu$ m, characterized by blunt ends, and conspicuous vacuoles. Both are illustrated by Sprague (1950). NR, EU.

Anthoxanthum odoratum L., sweet vernalgrass

Deuteromycotina – Hyphomycetes:

*Drechslera dematioidea* (Bubák and Wróbl.) Subr. and P. C. Jain – Collected at Butt Mt. lookout and transmittor area, Giles Co., El. 4100', N37° 22.140', W80° 37.403', June 15, 2004, R04-13. This fungus is a common cause of leaf spots and wilted leaves on *A. odoratum* (R and R 1996) but this is the first report from Giles Co.

#### Aristida spp., three-awn

- 1. Aristida dichotoma Michx., triple-awn grass
- 2. A. oligantha Michx., prairie three-awn
- 3. A. purpurascens Poiret, arrowfeather

All *Aristida* collections are from the Pembroke quarry, Rt. 623, Giles Co., El.1445', N37° 18.781', W80° 39.321', Oct. 22, 2006.

#### Ascomycotina:

*Physalospora* sp. – Large, black, erumpent, papillate ascocarps; no paraphyses; asci bitunicate; spores biseriate, hyaline, granular, ellipsoid, 25-35 X 8-9μm. On leaves of 1 (R06-32) and 3 (R06-31). Sprague (1950) listed four *Physalospora* spp. on grasses none of which fits these Giles Co. collections; the closest is *P. rhodina* (Berk. And Curt.) Cooke having spores measuring 24-42 X 7-17μm. This is now *Botryosphaeria rhodina* (Cooke) Arx (Farr et al. 1989). Either *P. rhodina* or *B. rhodina* is an uncertain choice for this fungus. NR, U.

## Deuteromycotina – Hyphomycetes:

Curvularia protuberata Nelson and Hodges – Found on incubated spikelets of 2, R06-30. Fits description and illustration by Ellis (1971); spores 4-septate, slightly curved, protruding hilum, measuring 27-35 X 10-14μm. It has been previously collected on 2 (R and R 1996), *Tripsacum* and *Paspalum* (R and R 1997), and *Eragrostis* and *Sporobolus* (Roane 2004).

Exserohilum halodes (Drechs.) Leonard and Suggs – Produced on incubated spikelets of 1 and 2; R06-32 and R06-31. Sometimes included in *E. rostratum* (Drechs.) Leonard and Suggs (Farr et al. 1989) but retained as a distinct species by Ellis (1971). 1 and 2. NR, U.

Fusarium graminearum Schwabe and F. poae (Peck) Wollenweb. – Produced on different incubated spikelets of 3, R06-31. Identified with the aid of keys and illustrations by Boothe (1971). NR, U.

Nigrospora sphaerica (Sacc.) E. Mason – Produced on incubated spikelets of 2 and 3; probably a saprophytic association. NR, U.

Periconia byssoides Pers. ex Mérat – Produced on incubated spikelets of **2**, R06-30. No doubt this fungus is saprophytic. For morphology, see Ellis (1971). NR, U.

## Deuteromycotina – Coelomycetes:

Colletotrichum caudatum (Sacc.) Peck – Produced on incubated spikelets of **2**, R06-30. This fungus was collected at the same site in 1995 (R and R 1996); it was listed under its synonym, *Ellisiella caudatum* Sacc.

*Phoma sorghina* (Sacc.) Boerma and VanKest. – Produced on incubated spikelets of 2, R06-30. NR, U.

## Arrhenatherum elatius (L.) Presl., tall oatgrass

Deuteromycotina – Coelomycetes:

Stagonospora arenaria (Sacc.) Sacc. – Collected on Butt Mt. in tower area, Giles Co., June 15, 2004, R04-11, El. 4200', N37° 22.14', W80° 37.403', previously collected on Augusta-Nelson Co. line; new for Giles Co.

## Arthraxon hispidus (Thunb.) Makino, small carpgrass

Ascomycotina:

*Mycosphaerella holci* Tehon – Collected at the parking area, Pandapas Pond, Montgomery Co., Sept. 4, 2005, R05-37, and along Peak Ck. at N.S. R.R. below Gatewood Dam, Pulaski Co., Aug. 19, 2007, R07-13, El. 2244', N37° 1.879', W80° 50.958'. Ascospores were 11-13 X 6-7μm. NR, U.

Deuteromycotina – Hyphomycetes:

Nigrospora sphaerica (Sacc.) E. Mason – Collected on foliage at the Pandapas Pond site above Sept. 4, 2005, R05-37; this is the first collection on this grass. It is illustrated and described by Ellis (1971). NR, U.

Deuteromycotina – Coelomycetes:

*Microsphaeropsis olivacea* (Bonord.) Höhn – A fungus on *A. hispidus* which keyed to *Coniothyrium* in several works (Barnett and Hunter 1998; Clements and Shear 1931; Sprague 1950; Stevens 1913) but to *Microsphaeropsis olivacea* (Bonord.) Höhn in Sutton's works (1973, 1980). It fits nicely into this taxon. Sutton (1980) lists a broad range of hosts, *Paspalum distichum* being the only grass host. This collection, R07-13, came from the Peak Ck., Pulaski site above. Spores were brown 1-celled, oval to ellipsoid, smooth, measuring 4-6 X 3-4μm. NR, U.

## Arundo donax L., giant reed

Deuteromycotina – Hyphomycetes:

Bipolaris zeicola (G. L. Stout) Shoem. – One colony was detected in a collection on foliage of plants from the Virginia Tech Horticultural Garden, Blacksburg, Montgomery Co., Oct. 7, 2007, R07-28, El. 2166', N37° 13.188', W80° 25.466'. B. zeicola is a common maize leaf-blighting fungus. See comments under Eragrostis spp. NR, U.

*Nigrospora sphaerica* (Sacc.) E. Mason – In the same collection at the site above, R07-28, on the same foliage. NR, U.

Avena sativa L., oats

Deuteromycotina - Other:

Rhizoctonia solani Kühn = Thanatephorus cucumeris (A. B. Frank) Donk – Found on a Plant Clinic specimen (Pl. Cl. 07-353) from Orange Co., May 16, 2007, causing sharp eyespot of cereals, R07-1. The identification of *Rhizoctonia* spp. on grasses is difficult. See discussion by R and R 1994, p. 284. This is the first report of *R. solani* on oats in a commercial field in Virginia; it had been found only in experimental plantings.

Bouteloua curtipendula (Michx.) A. Gray, side-oats grama

One colony of *B. curtipendula* was visited; all entries below are from that colony. The site is on a shelf about 0.2 mi W. of Rt. 660, N. of N.S. R.R., Montgomery Co., El. 1845', N37° 9.548', W80° 28.310', collected Sept. 8, 2005, R05-40. None of the fungi identified correlated with leaf spots.

Deuteromycotina – Hyphomycetes:

*Bipolaris zeicola* (Stout) Shoem. – Brown conidia measuring 44-88 X 6-10μm, 6-10 septa. A frequent pathogen of maize. NR, U.

*Nigrospora sphaerica* (Sacc.) E. Mason – Emerged on incubated leaves and stems; no doubt, saprophytic. NR, U.

Periconia atra Cda. – On incubated leaves and stems. Illustrated and described by Ellis (1971). NR, U.

Deuteromycotina – Coelomycetes:

Amerosporium atrum (Fkl.) Höhn – On incubated material; illustrated by von Arx (1981). Spores measured 7-9 X 2µm. NR, U.

*Heteropatella* sp. – A fungus keying to *Heteropatella* sp. with 1-3- (mostly 3-) septate conidia measuring 33-41 X 5-6 $\mu$ m (Sutton 1980). Most species of this genus are not broader than 4 $\mu$ m. This collection fits fairly well into *H. antirrhini* Buddin and Wakefield but that taxon is a highly unlikely to be a grass fungus.

Brachyelytrum erectum (Schreb.) Beauv., long-awned wood grass

Deuteromycotina – Coelomycetes:

Stagonospora brachyelytri Greene – Collected on Taylor's Hollow Road, Rt. 712, 1.3 mi. From Rt. 723, July 17, 2005, R05-18, and Oct. 1, 2006, R06-22, Montgomery Co., El. 1505', N37° 12.418', W80° 21.035'. This fungus has been collected from three other counties (R and R 1996), in each case it appeared to cause spectacular, elongated leaf spots. Spores measured 30-35  $\times$  5-6 $\mu$ m, were 1-3-septate, constricted at septa, rounded both ends. See Sprague (1950) for a description.

Amerosporium atrum (Fkl.) Höhn – Produced on incubated spikelets of 3, R06-31. This fungus has been collected on *Cynodon* and *Danthonia* (R and R 1996), and *Glyceria, Leersia*, and *Muhlenbergia* (R and R 1997), but not on *Aristida*. Illustrated by von Arx (1981). NR, U.

## Bromus spp., brome grass

- 1. Bromus inermis Leyss., smooth brome.
- 2. B. pubescens Willd. including B. purgans L., Canada brome.
- 3. B. racemosus L., racemose brome.
- 4. B. sterilis L., barren brome.

See Harvill et al. (1992) for synonomy.

## Ascomycotina:

Blumeria graminis (DC) E. O. Speer (formerly Erysiphe graminis DC) – The cause of powdery mildew on grasses has a broad host range. This collection, R07-20, was sent to the Plant Clinic (Pl. Cl. 07-384) on 1 from Shenandoah Co., May 17, 2007. NR, EU.

*Paraphaeosphaeria michotii* (West.) O. Erikss. – Found on inflorescences of a senescent roadside colony of 1 at the Pembroke quarry, Rt. 623, Giles Co., El. 1660', N37° 18.761', W80° 39.401', Sept. 2, 2007, R07-20. It is described and illustrated by S and B (1985), and E and E (1985). It has been collected on several grasses but no *Bromus* spp. (R and R 1996; Roane 2004). Ascospores are dark brown, constricted at the two septa, rounded both ends, measuring 16-24 X 4-5μm. See notes on *Coniothyrium* below. NR, U.

*Phaeosphaeria nodorum* (E. Müller) Hedj. – Present on inflorescences of **1** in collection above, R07-20. It is described and illustrated by S and B (1989), and E and E (1985). Ascospores were 5-septate, measured 22-25 X 4-5 $\mu$ m. It has been found on **1** in several northcentral states. NR, EU.

Phaeosphaeria luctuosa (Niessl) Otani and Mikawa — Collected on senescent foliage of **2**, behind upper cabin at Claytor L. State Pk., edge of woods, Pulaski Co., El. 1860', N37° 3.105', W80° 37.51', Oct. 15, 2007, R07-29. It is described and illustrated by S and B (1989), and E and E (1985). Note: *B. purgans* was the original identity of this collection. This taxon is now included in *B. pubescens* (Harvill et al. 1992). NR, U.

## Deuteromycotina – Hyphomycetes:

Periconia atra Cda. – Appeared on incubated spikelets of 3 collected Oct. 1, 2006, at the Falls Ridge Nature Conservancy property, Fagg, Montgomery Co., El. 1495', N37° 11.523', W80° 31.166', R06-26. *P. atra* is described and illustrated by Ellis (1971). NR, U.

*Periconia lateralis* Ellis and Everh. – Produced on incubated spikelets of **2** collected along Walker Ck., Rt. 708 near Rt. 622, Giles Co., El. 1634', N37° 17.777', W80° 42.387', Oct. 22, 2006, R06-36. The fungus is described and illustrated by Ellis (1971). NR, U.

## Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wilson. – On 4 collected June 10, 2007, at Ironto, Montgomery Co., along N.S. R.R. E. of m.p. 266, El. 1298', N37° 13.470' W80° 16.334', R07-5. This is a well-known fungus on many grasses. It was found in 1990 on 4 (R and R 1996).

Coniothyrium sp. – A fungus producing small (5 X 2-3 $\mu$ m), brown pycnidiospores appeared on incubated spikelets collected on 1 at the Pembroke quarry site above, R07-

20 S and B (1985) allude to C. scirpi = C. zeae as being the anamorph of P. michotii. The spores in this collection were too small to be accommodated by this taxon.

Stagonospora bromi A. L. Smith and Ramsb. – Appeared on incubated inflorescenses of 1 collected at the Pembroke quarry site above, R07-20. The fungus was found on 1 in Montgomery Co. in 1955 (R and R 1996). See Sprague (1950) for a description. New only for Giles Co.

## Calamagrostis spp., reedgrass

- 1. Calamagrostis X acutiflora (Schad.) Rehb. var. 'Karl Foerster', a patented Hort. var. of feather reedgrass.
- 2. C. arundinacea Roth, Foerster's feather reedgrass.
- 3. C. porteri Gray, no common name.

#### Ascomycotina:

Phyllachora graminis (Pers.:Fr.) Nitschke – The tar spot fungus was prevalent on lower foliage of 1 collected at the Virginia Tech Horticultural Garden, Blacksburg, Montgomery Co., Oct. 7, 2007, El. 2100', N13.188', W80° 25.465', R07-26. P. graminis is unreported on Calamagrostis spp. in Virginia (Farr et al. 1989), but was rampant on the lower half of all plants of 1 in the Garden. NR, U.

#### Deuteromycotina - Hyphomycetes:

Curvularia inaequalis (Shear) Boedijn – Collected on 3 growing among rock outcrops at the towers, Butt Mt., Giles Co., El. 4200', N37° 22.137', W80° 37.413', Aug. 17, 2004, R04-34. Conidia were both straight and curved, 3-4-, mostly 4-septate, measuring 30-31 X 9-11µm. In 5-celled spores, the third cell was enlarged. Farr et al. (1989) do not list this host. NR, U.

## Deuteromy cotina-Coelomy cetes:

Ascochyta graminea (Sacc.) Sprague and A. G. Johnson – Collected on 3 at the Butt Mt. site above, Apr. 24, 2004, R04-2. Spores measured 14-17 X 4-5 $\mu$ m. See note in next entry. NR, U.

Ascochyta sorghi Sacc. – Collected on 2 at the Community Arboretum, Va. Western Community College, Roanoke, Roanoke Co., El. 1094' N37° 14.695', W79° 58.455', Sept. 5, 2004, R04-43, and Sept. 9, R04-48. Sprague (1950) shows *A. sorghi* spores to be slenderer (11-21 X 1.6-4.0μm) than *A. graminea* (11-20 X 3.9-9.0); Punithalingam (1979) shows *A. sorghi* to measure 16-20 X 6-8μm and *A. graminea* 14-15 X 4-5μm, or virtually reverse to those of Sprague. I cannot resolve these differences so I have assigned these taxa as described by Sprague (1950). NR, U.

Colletotrichum graminicola (Ces.) G. W. Wilson – This fungus occurred on all three Calamagrostis species at all three locations. On 1, 2. NR, U.

Pseudoseptoria obtusa (Sprague and A. G. Johnson) Sutton – Found on overwintered culms and foliage of 3 at the Butt Mt. site, R04-2. Pycnidiospores were lunate, 22-25 X 4-5μm, with blunt ends, enclosing a single oil globule. While this fits the description of *P. obtusa* in Sprague's (1950) book, it also seems out of place; most of his records are from western grasses and the easternmost collection is from North Dakota. On the other hand, in Virginia *C. porteri* grows in high altitudes amid Canadian flora. NR, U.

Septoria oudemansii Sacc. – Collected on 3 at the Butt Mt. site Aug. 17, 2004, R04-34. Although having 1- and 3-septate pycnidiospores as described by Sprague (1950), this fungus seems to be a misfit for Septoria. Spores measured 13-14 X 3.0-3.5μm for 2-celled, and 12-16 X 3-4μm for 4-celled. NR, U.

Stagonospora nodorum (Berk.) Cast. and Germ. – Collected on 2 Sept. 2 (R04-43) and Sept. 9, 2007 at the Roanoke Arboretum site above. On foliage, pycinidiospores are mostly 3-septate, measuring 15-20 X 2-4 $\mu$ m. This fungus causes leaf spots, glume blotch, and node rot of *Triticum* in Virginia and has been found on several other grasses (R and R 1994, 1996, 1997; Roane 2004). NR, U.

*Wojnowicia hirta* Sacc. – Collected on **3** at Butt Mt. site above. Pycnidia on overwintered culms; spores yellow-brown, usually 7-septate, 35-40 X 4-5 $\mu$ m; a weak pathogen on grasses (Sprague 1950). NR, U.

Chasmanthium latifolium (Michx.) Yates = Uniola latifolia Michx., wild oats

## Ascomycotina:

Phaeosphaeria eustoma (Fkl.) L. Holm – Occurring on incubated leaves collected along Walker Ck. on Rt. 708 near Rt. 622, Giles Co., Oct. 22, 2006, R06-33, El. 1634', N37° 17.777', W80° 42.387'. From ascospore measurements, 13-16 X 5-6μm, 3-septate, this fungus fits *P. eustoma* but like collections on *Leersia virginica* (Roane 2004, p. 149), the cells contained a globoid vacuole or oil globule. This character is not noted in available descriptions of *P. eustoma* (S and B 1989). Thus, this collection is dubiously placed in *P. eustoma*. NR, U.

## Deuteromycotina – Hyphomycetes:

Fusarium semitectum Berk. and Ravn. – Produced on incubated stems and leaves. Not known to inhabit grasses; more than likely a saprophyte. Collected at the Walker Ck. site above, R06-33. Identified from keys of Nelson et al. (1983).

Nigrospora sphaerica (Sacc.) E. Mason – Appeared on incubated leaves, stems, and spikelets collected at the Walker Ck. site above (R06-33). NR, U.

*Periconia atra* Cda. – Appeared on incubated leaves collected at the Walker Ck. site above (R06-33). Identified from keys of Ellis (1971). NR. U.

*Tricothecium roseum* (Pers.:Fr.) Link – Appeared on incubated stems collected at the Walker Ck. site above. It causes a pink head mold of sorghum and wheat. It is described and illustrated by Kendrick and Carmichael (1973), Carmichael et al. (1980), von Arx (1981), and E and E (1985). In older literature it is listed at *Cephalothecium roseum* (Stevens 1913; Heald 1933). NR, U.

## Chloris verticillata Nutt., windmill grass

Collections were made from a colony of windmill grass growing in a traffic island at Avenham Ave. and Franklin Rd. in Roanoke, Aug. 17, 1994 (R and R 1996). The site was revisited on Sept. 9, 2004, R04-49, and some new fungi were found.

## Deuteromycotina – Hyphomycetes:

*Bipolaris spicifera* (Bainier) Subr. – Appeared on incubated leaves and stems; conidia were cylindrical with rounded ends, consistently 3-septate, measuring 19-30 X 8-10μm, end cells lighter colored than the two central cells. It was also found in the

1994 collection (R and R 1996). Ellis (1971) makes no mention of cell color differences (under *Drechslera spicifera*) in four-celled species; this is more commonly a trait of *Curvularia* spp. They also list *Chloris* spp. as a host of *D. australiensis* (= *B. australiensis*) which is similar to *B. specifera* but *D. australiensis* conidia are more elliptical and are 3-5 septate.

Fusarium oxysporum Schlecht.:Fr. – On incubated leaves. Identification was facilitated by the keys of Booth (1971) and Nelson, et al. (1983). F. oxysporum typically colonizes roots of various hosts; occurrence on aerial plant parts suggests it functioned as an saprophyte on C. verticillata. NR, U.

*Idriella lunata* Nelson and Wilhelm – Sporulated on incubated leaves and stems. The one-celled, lunate, hyaline conidia measured 11-13 X  $2\mu m$ . This fungus is a cause of strawberry root rot. Thus, its identity on *Chloris* must be acepted with caution. NR, U.

## Cinna spp., woodreed

- 1. Cinna arundinacea L., stout woodreed.
- 2. C. latifolia (Goepp.) Grisebach, drooping woodreed.

#### Ascomycotina:

*Phaeosphaeria nigrans* (Rob. ex Desm.) L. Holm – Occurred on 1 collected along wooded road behind barns at Glen Alton, Giles Co., Sept. 20, 2005, El. 2620', N37° 26.035', W80° 32.736', R05-45 and was collected on 2 off Rt. 725 at confluence of Toms and Poverty Creeks, Montgomery Co., Aug. 14, 2005, El. 1785', N37° 13.937', W80° 31.438', R05-34. *P. nigrans* is characterized by yellow brown 5-septate ascospores, the penultimate cell enlarged, measuring 17-24 X 3-4μm. It was collected previously in Washington (S and B 1989). NR, EU.

## Cynodon dactylon (L.) Pers., Bermudagrass

Deuteromycotina – Hyphomycetes:

*Bipolaris cynodontis* (Marig.) Shoem. – Collected at Montgomery Tunnels, Montgomery Co., June 11, 2004, R04-09. Conidia measure 30-75 X 10-16 $\mu$ m (commonly 50 X 13 $\mu$ m), 3-9-septate (7-8) (Ellis 1971). This is a common leaf spotting fungus on *C. dactylon*.

Bipolaris sorokiniana (Sacc.) Shoem. – Occurred on specimens sent to the Plant Clinic (Pl. Cl. 04-1306) from the Virginia Beach Co. Agt., Oct. 21, 2004, R04-61. Spores measured 40-120 X 17-28μm (commonly 60-100 X 18-23μm), were 3-12-septate, dark brown (Ellis 1971). This is a common graminicolous fungus but has not been found on Bermudagrass in eastern U.S.A. before. NR, EU.

Bipolaris spicifera (Bainier) Subr. – Occurred on collections from the Montgomery Tunnels site above. Conidia measured 20-40 X 9-14 $\mu$ m (commonly 30-36 X 11-13 $\mu$ m) were consistently 3-septate (Ellis 1971).

Curvularia intermedia Boedijn – Occurred on the Pl. Cl. 04-1306 collection above. It is illustrated and described by Ellis (1971). Its was reported previously in Alabama on Bermudagrass. NR, V.

Dactylis glomerata L., orchardgrass

#### Ascomycotina:

Claviceps purpurea (Fr.:Fr.) Tul. – Collected along Rt. 666 W., Burkes Garden, Tazewell Co., July 10, 2005, R05-15, El. 3082', N37° 6.418', W81° 21.514'. This collection merely extends the known range of orchardgrass ergot in Virginia.

## Deuteromycotina – Hyphomycetes:

Cercosporidium graminis (Fkl.) Deighton – The cause of leaf streak, this collection was sent to the Plant Clinic (Pl. Cl. 06-67) from Cumberland Co., Feb. 22, 2006, R06-2. It was causing leaf blades to wilt from tips downward in overwintering foliage. It is common on *D. glomerata*.

Drechslera dictyoides (Drechs.) Shoem. – This collection was sent to the Plant Clinic from Pennington Gap, Lee Co. (Pl. Cl. 06-395), May 15, 2006, associated with large areas of dead plants, R06-3. Conidia measured 100 X 15-17μm, had 6-11 septa; they may be as long as 240μm (Ellis 1971). This fungus frequently damages foliage of Festuca elatior (tall fescue) in Virginia but is previously unrecorded on D. glomerata (Farr et al. 1989). NR, U.

Fusarium roseum Link:Fr. – This fungus appeared on incubated leaves of plants sent to the Plant Clinic on Aug. 10, 2004 (Pl. Cl. 04-961) from Prince Edward Co., R04-53. Farr et al. (1989) list three Fusarium spp. on Dactylis but none had conidia matching the morphology of Fusarium in this collection. F. roseum provided the best match (Booth 1971; Nelson et al. 1983). NR, U.

*Nigrospora sphaerica* (Sacc.) Mason – appeared on incubated leaves of collection R04-53 above and on Pl. Cl. 04-1324 specimen from Pennington Gap, Lee Co., R04-62. It had been identified only once before on *Dactylis* (R and R 1996).

Rhynchosporium orthosporum Cald. – This specimen came from a field in Shenandoah Co. (Pl. Cl. 07-790) causing scald on about 80% of plants in a 10-acre field. The fungus has been found previously on orchardgrass in several locations (R and R 1996) but no such damaging occurrence has been observed, R07-11.

## Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wils. – Was present on leaves of specimens R04-53 and –62 sited above. It appeared to be causing death of some plants in the Lee Co. sample, and spotting and dying of leaves in the Prince Edward Co. sample. *C. graminicola* is saprophytic on many grasses but is sometimes an aggressive pathogen.

Danthonia compressa Austin, mountain oatgrass

## Basidiomycotina:

Ustilago residua G. P. Clinton – Cause of panicle smut, this specimen was collected at the Butt Mt. towers site, Giles Co., El. 4200', N37° 22.140', W80° 37.403', June 15, 2004, R04-08. Spores measure 5-7 X 7-10 $\mu$ m, are prominently verrucose, mostly globose. It has been collected before on *D. compressa* and *D. spicata* (R and R 1996). Smutted inflorescences and spores are illustrated by Fischer (1953).

Deuteromycotina – Hyphomycetes:

Curvularia inaequalis (Shear) Boedijn – Collected at the Butt Mt. site above, April 22, 2004, R04-3. This fungus has been collected once before on Aristida oligantha (R and R 1996) but is new for Danthonia spp. Conidia are relatively straight, measure 36-42 X 9-13μm, 3-4-septate, middle cell is widest in 5-celled conidia. Sprague (1950) describes it well, Ellis (1971) illustrates it. NR, U.

Another *Curvularia* also fruited in this collection; conidia had protruding hyla, measured 38-47 X 9-12µm, were 2-4-septate. It keyed out to *C. cymbopogonis* (Ellis 1971). It has been reported previously only on *Rottboellia cochinchinensis* in Louisiana (Farr et al. 1989). It seems unlikely that a fungus of tropical habitat would be present among Canadian flora in Virginia. However, it was identified on collections from *Bothriochloa* before (Roane 2004). NR, U.

Myrothecium verrucaria (Albertini and Schwein.) Ditmar:Fr. – This fungus fruited in a collection from the Butt Mt. site above, R04-3. Conidia were 1-celled, measured 10 X 3μm, most possessed two conspicuous vacuoles and perfectly fitted the description and illustrations by Ellis (1971). It was found on Zoysia in a collection from James City Co. in 2003 (Roane 2004). NR, U.

Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wils. – Collected at the Butt Mt. site above, R04-3, this is only the second collection of this fungus on *D. compressa* in eastern U.S.A. (R and R 1996); unusual for such an ubiquitous species on a widespread host.

## Deschampsia flexuosa (L.) Beauv., hairgrass

Ascomycotina:

*Paraphaeosphaeria michotii* (West.) O. Eriks. – Collected at the Butt Mt. site above, June 15, 2004, R04-10. This fungus is characterized by brown, 3-celled ascospores biseriately arranged in the ascus, constricted at the septa, rounded ends, measuring 16-17  $\times$  4-5  $\mu$ m. It is described and illustrated by E and E (1985), and S and B (1985).

Deuteromy cotinia-Hyphomy cetes:

*Bipolaris spicifera* (Bainier) Subr. – Collected at the Butt Mt. site above, June 15, 2004, R04-10. See under *Chloris* for discussion. NR, U.

Deuteromycotinia – Coelomycetes:

*Colletotrichum graminicola* (Ces.) G. W. Wils. Also from the Butt Mt. collection above, June 15, 2004, R04-10, this common fungus has not been previously associated with *Deschampsia* spp. (Farr et al. 1989). NR, U.

## Dichanthelium spp.

- 1. D. boscii (Poir.) Gould and Clark, no common name.
- 2. D. clandestinum (L.) Gould, deer tongue.
- 3. D. sphaerocarpon (Ell.) Gould, round fruited panic.

Ascomycotina:

*Phyllachora punctum* (Schwein.) Orton and Stevens – Collected on **2** at two Montgomery Co. locations: Between Rt. 683 and N.S. R.R., Walton, El. 1824', N37° 9.176', W80° 31.007', Aug. 2, 2005, R05-29. Road from parking area to Pandapas Pond, El. 2216', N37° 16.966', W80° 28.207', Sept. 4, 2005, R05-39. Ascospores measured 11-13  $\times$  6-8μm; spermatia numerous as described by Orton (1944). Although reported from other *Dichanthelium* spp., this is my first report on deer tongue. It is listed by Farr et al. (1989) from Virginia.

Deuteromycotina – Hyphomycetes:

Curvularia lunata (Wakk.) Boedijn – Occurred on leaves of 3 collected Aug. 15, 2004, R04-31, 1/8 mi below Gatewood dam, on Peak Ck., Pulaski Co. Although common on other grass species in Virginia (Farr et al. 1989; Roane 2004; R and R 1994, 1996), including *Dichanthelium* spp., it has not been found on 3 before. The specimen was inadequate for an herbarium. NR, U.

Nigrospora sphaerica (Sacc.) E. Mason – Collected on **3** at the south end of the bridge at Pandapas Pond, Montgomery Co. Sept. 4, 2005, El. 2106', N37° 16.926', W80° 27.951', R05-35. This fungus has not been found on any *Dichanthelium* spp. before (Farr et al. 1989). NR, U.

Tetraploa aristada Berk. and Broome – Collected on 3 at the Pulaski site above. R04-31. Although according to Farr et al. (1989) *T. ellisii* has been recorded more often in temperate North America, this specimen fits *T. aristata*. It is illustrated by Ellis (1971) and E and E (1985). NR, U.

Deuteromycotina - Coelomycetes:

Cylindrosporium glyceriae Ell. and Everh. – was collected on 3 at the Pulaski Co. site above, R04-31. The slender conidia were 0-3-septate, measured 15-28 X 2.0-2.5µm. It is described by Sprague (1950). NR, U.

Stagonospora simplicior Sacc. and Briard – A cause of leaf spots on several Dichanthelium spp., this fungus is easily identified by its broad, vacuolate, pycnidiospores (see Sprague, 1950). It was collected on 1 off the Forest Service road ½ mile W. of U.S. 460 on Gap Mt., Montgomery Co., July 20, 2005, El. 2446', N37° 16.740', W80° 29.011', R05-21; on 2 upstream from entry bridge at Glen Alton, Giles Co., Sept. 20, 2005, El. 2577' N37° 25.798', W80° 33.065', R05-46; on 3 at south end of long bridge at Pandapas Pond site above, R05-35. It has been collected before on 1 and 2 but not 3. On 3, NR, V.

## Digitaria filiformis (L.) Koel., fingergrass

Basidiomycotonia – Uredinales:

Puccinia substriata Ellis and Barth. – Collected at the Butt Mt. Tower site, Giles Co., Aug. 17, 2004, El. 4200' N37° 22.137', W80° 37.413', R04-36. This rust fungus was determined by eliminating those rusts listed on *Digitaria* spp. by Farr et al. (1989) which did not fit the descriptions given by Cummins (1971); only the *P. substriata* description fit. Urediniospores were ellipsoid to obovate, echinulate, 2-4-pored, measuring 26-35 X 26-33μm. Scarce, pedicellate teliospores measured 31-44 X 20-

24μm. The northermost occurrence on *Digitaria* spp. in our region is on *D. cognata* = *Leptoloma cognatum* in North Carolina (Farr et al. 1989). NR, V.

Deuteromy cotina-Coelomy cetes:

Stagonospora carcinella Brun. – Collected at the Butt Mt. towers site above, R04-36. This fungus is listed on Carex and Juncus by Farr et al. (1989). Spores measured 13-17 X 5-6 $\mu$ m, were 3-celled, guttulate, fusiform, rounded but sometimes pointed at end. It is described and illustrated by Sutton (1980). Several graminicolous fungi are hosted by sedges and rushes. NR, U.

## Echinochloa crusgalli (L.) Beauv., barnyardgrass

Deuteromycotina – Hyphomycetes:

Pyricularia grisea (Cooke) Sacc. – Collected at N.S. R.R. and Rt. 661 (Crismond Mill Rd.), Christiansburg, Montgomery Co., Sept. 3, 2006, El. 1922', N37° 8.849', W80° 26.695', R06-15. Common on Digitaria and Setaria spp., this fungus causes gray leaf spot of *E. crusgalli*. Spores were typical of those described and illustrated by Ellis (1971). NR, EU.

## Eleusine indica (L.) Gaertn., goosegrass

Deuteromycotina – Hyphomycetes:

*Bipolaris cynodontis* (Marig.) Shoem. – Collected on Crab Ck. side of N.S. R.R. between Rt. 663 and R.R., Montgomery Co., July 20, 2004, El. 1740', N37° 9.175', W80° 31.01', R04-26. Conidia were mostly 5-8-septate, measured 42-56 *X* 12-14μm. A cause of leaf spot on *Cynodon dactylon*, it is widespread in Virginia but this is the first collection on goosegrass outside of Florida. NR, V.

Myrothecium carmichaelii Grev. – Occurred on incubated leaves collected from site R04-26 above. M. roridum is more likely to be found here but the conidial dimensions,  $11-12 \times 2\mu m$ , set it aside from M. roridum (6-8  $\times 1.5-2.5\mu m$ ). Ellis (1971) in side by side illustrations clearly distinguishes the two species. However, Farr et al. (1989) do not list M. carmichaelii and Ellis does not list a grass host. NR, U.

## Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wils. – This was the predominant fungus in this collection, R04-26. This collection merely extends the known range of the association.

*Phoma sorghina* (Sacc.) Boer., Doren., and VanKester. – Present on foliage from site R04-26 above. Spores measured 5-7 X 2-3μm. It keyed to *Phyllosticta sorghina* (Synonym of *Phoma sorghina*) in Sprague's (1950) keys. NR, U.

## Elymus spp., wild rye

- 1. Elymus riparius Wieg., river bank wild rye
- 2. E. virginicus L., Virginia wild rye

Basidiomycotina – Uredinales:

Puccinia montanensis Ellis – Collected on 1 along Walker Ck., Rt. 708, above bridge on Rt. 622, Giles Co., El. 1634', N37° 17.777', W80° 42.387', Oct. 22, 2006, R06-37. Pedicels on teliospores were absent or very short; however, no paraphyses were observed. Teliospores were photographed, to be included with specimen. Spores matched well with those shown by Cummins (1971). The fungus usually occurs on western Elymus spp., but is known in Kentucky and West Virginia. NR, V.

#### Ascomycotina:

*Phaeosphaeria eustoma* (Fkl.) L. Holm – Collected on 1 at two locations: R04-33, along the road to Gatewood Dam, Pulaski Co., Aug. 15, 2004, El. 2192', N37° 2.341', W80° 50.07', R04-32; at Falls Ridge at edge of field between parking area and waterfall, Fagg, Montgomery Co., Aug. 20, 2006, El. 1495', N37° 11.523', W80° 31.166', R06-11. The 3-septate spore dimensions on R06-11 fit well within those published, 20-30 X 7-8μm (Ellis 1985); those from R04-32 had the proper morphology, but were smaller, 13-16 X 3-4μm and this morphology did not match any other 4-celled *Phaeosphaeria*. NR, U.

*Phaeosphaeria nigrans* (Rob. ex Desm.) L. Holm. – Collected on spikes of 1 at the Falls Ridge site, R06-25, above, Oct. 1, 2006. Ascospores measured 20-28 X  $5\mu m$  were mostly 6-celled, the penultimate cell larger than others. The fungus fruited on incubated florets; illustrated by Ellis and Ellis (1985) and S and B (1989). NR, U.

*Phyllachlora graminis* (Pers.:Fr.) Nitschke – The cause of tar spot, common on *Elymus* spp., this fungus was found on 1 along Walker Ck., Giles Co., site R06-37 above; at the Falls Ridge site R06-11 above; along the road to Gatewood Dam, Pulaski Co. site R04-32 above; at Rt. 600 and 1<sup>st</sup> N.S. R.R. underpass, Parrott, Pulaski Co., El. 1688', N37° 12.197', W80° 36.600', Nov. 2, 2007, R07-34. This last collection had been colonized by *Fusarium sambucinum* Fkl. (R and R 1997, p. 22); on 2 at Roane's Wharf, Gloucester Co. off Rt. 686, July 23, 2005, El. 5', N37° 21.902', W76° 27.783', R05-22. *P. graminis* is encountered in Virginia wherever *Elymus* spp. grow.

## Deuteromycotina – Hyphomycetes:

*Bipolaris cynodontis* (Marig.) Shoem - Fruited on senescent leaves of 1 from site R07-34 above. Conidia measured 45-55 X 12-15μm were 6-9-, mostly 6-septate; the morphology fitted published descriptions very well (Sprague 1950; Ellis 1971). It has been found only once previously in Virginia (Roane 2004).

*Bipolaris sorokiniana* (Sacc.) Shoem. – Also fruited on leaves of **1** from the Pulaski Co. site above, R07-34. Conidia are much larger and darker than those of *B. cynodontis*, measuring 85-105 X 17-21 $\mu$ m, mostly 9-septate. It has been found previously on 1 in Virginia. (R and R 1997).

Fusarium moniliforme J. Sheld. – Occurred on incubated spikelets of 1 collected at site R06-25 above. This is a common maize pathogen that also is facultative on many plants. It was found in an effort to seek seed-borne fungi. NR, U.

Fusarium roseum Link:Fr. – Also occurred on spikelets of 1 in the same collection, R06-25. Fusarium species were identified with the aid of keys by Boothe (1971) and Nelson et al. (1983). NR, V.

Nigrospora sphaerica (Sacc.) E. Mason – Occurred on foliage of 1 collected along Walker Ck., Oct. 22, 2006, site R06-37 above. It had been collected previously in Montgomery Co. (R and R 1997).

Periconia atra Cda. – Found on incubated spikelets and foliage of 1 at site R06-37 above. It is characterized by tight terminal clusters of brown, spherical, conidia measuring 5-9µm diameter (Ellis 1971). Probably saprophytic. NR, U.

Periconia lateralis Ellis and Everh. – Characterized by a terminal, sterile projection of the conidiophore, with brown, spherical conidia measuring 8-15 μm produced laterally, subterminally. Collected on 1 at three locations: Along Walker Ck., Giles, Oct. 22, 2006, R06-37, on incubated spikelets and foliage; at Falls Ridge, Montgomery Co., Oct. 1, 2006, on incubated spikelets R06-25, and foliage Aug. 20, 2006, R06-11; at Parrott, Pulaski Co., R07-34, on incubated foliage. Ellis (1971) lists several grass hosts, but Farr et al. (1989) list only two. NR, U.

Deuteromycotina – Coelomycetes:

Collectotrichum graminicola (Ces.) G. W. Wils. - On culms and leaf sheaths of 1 collected at Parrott, Pulaski Co., Nov. 2, 2007, R07-34. This merely extends the range of this association in Virginia.

Stagonospora nodorum (Berk.) Cast. and Germ. – This is the glume blotch fungus of wheat that also occurs on many other grasses. It was found on 1 along the road to Gatewood Dam, Pulaski Co., Aug. 15, 2004, R04-32. Farr et al. (1989) list several *Elymus* spp. as hosts but not *E. riparius*. NR, U.

## Eragrostis spp., lovegrasses

- 1. Eragrostis cilianensis (All.) Lutate, stinkgrass.
- 2. E. pectinacea (Michx.) Nees, tufted lovegrass.
- 3. E. pilosa (L.) Beauv., India lovegrass.
- 4. E. spectabilis (Pursh.) Steudel, purple lovegrass.

## Basidiomycotina – Ustilaginales:

Ustilago spermophora Berk. and Curtis – Causing seed smut, characterized by enlargement of scattered ovaries into sori 1-2 mm diam. Collected on 1 in a field, N.E. corner of Clay and Jefferson St., Blacksburg, Montgomery Co., Oct. 7, 2004, R04-57, El. 2170', N37° 13.97', W80° 24.02', and on 2 between N.S.R.R. and Rt. 663, Crab Ck. side, Walton, Montgomery Co. Aug. 14, 2004, R04-33, El. 1740', N37° 9.173', W80° 31.01'. In each case, 1% or less of spikelets were infected. It has been collected previously on 1 in Montgomery Co. (R and R 1997) and on 2 in Giles and Pulaski Cos. (Roane 2004). This is a first report for this association on 2 in Montgomery Co.

Deuteromycotina - Hyphomycetes:

*Bipolaris cynodontis* (Marig.) Shoem. – Collected on **2** at the Walton, Montgomery site above, R04-33. Conidia were 5-8-septate,  $34-70 \times 10-17 \mu m$ , as depicted by Ellis (1971). This is the first collection in Montgomery Co. but was collected in Pulaski Co. (R and R 1997).

*Bipolaris nodulosa* (Berk. and Curtis) Shoem. – Causing leaf spots on 1 in Roane's Garden, 607 Lucas Dr., Blacksburg, Montgomery Co., Sept. 2, 2004, R04-42, El. 2167', N37° 14.465', W80° 24.686'. Conidia were 4-9-septate, measured 45-61 X 14-16μm,

slightly narrower than published by Ellis (1971) but conforming to width published by Sprague (1950). This is a new record on 1 only in Montgomery Co.

Bipolaris sorokiniana (Sacc.) Shoem. – Also collected on 1 from Roane's garden site above, but on Sept. 11, 2006, R06-18. Conidia were dark brown, 5-9-septate, 50-105 X 17-24μm, well within published dimensions (Ellis 1971; Sprague 1950). Although the fungus has a very broad host range, this is the first report on 1 in eastern U.S.A. NR, EU.

Bipolaris zeicola (Stout) Shoem. – Collected on 1 from Roane's garden, Sept. 11, 2006 site R06-18. Although first described on maize by Stout (1930), this fungus remained obscure until 1941, when Ullstrup (1944) rediscovered it and named it Helminthosporium carbonum. With 6-12 septa, conidia measure 30-100 X 14-16μm (Ellis 1971; Sprague 1950) may be dark brown, more cylindrical than for B. sorokiniana with which it may be confused. This is the first collection on 1. NR, U.

Curvularia lunata (Wakker) Boedijn – This fungus occurred on senescing leaf tips of 3 in a parking area between New R. and N.S.R.R., Glen Lyn, Giles Co., July 31, 2005, El. 1510' N37° 22.251' W80° 28.107'. Conidia were asymmetrical, 3-septate, measuring 18-24 X 8-10 $\mu$ m, end cells hyaline, others dark. Fits well with description by Ellis (1971); known on other *Eragrostis* spp. but not on 3 (R and R, 1997). NR, U.

Curvularia protuberata Nelson and Hodges – Collected on 3 at Walton, Montgomery Co. in a parking area between N.S.R.R. and Rt. 683, creek side, El. 1824', N37° 9.176', R05-28, Aug. 2, 2005. Conidia with protruding hila, were 4-septate, end cells mostly hyaline, measuring 27-35 X 10-14μm. Not reported on 3 before. NR, U.

Found on incubated spikelets of 4 from the Pembroke quarry, Giles Co., El. 1655', N37° 18.781', W80° 39-321', R06-27, Oct. 8, 2006. Conidia as in R05-28 above. New association for 4. NR, U.

Exserohilum halodes (Drechs.) Subr. and Jain – Collected on 1 at Roane's garden site R06-18 above, Sept. 11, 2006. Conidia cylindrical to ellipsoidal, with protruding hila, measured 68-75 X 16-19 $\mu$ m, had 6-8 septa, characterized by a dark septum for each end cell (Ellis, 1971). NR, U.

Exserohilum monoceras (Drechs.) Leonard and Suggs – Collected on 3 at the Walton site above, Aug. 2, 2005, R05-28. Conidia were more fusiform than those of *E. halodes* above, measuring 70-110 X 15-20μm, were 6-9 septate. NR, U.

Fusarium avenaceum (Fr.:Fr.) Sacc. – Collected on 1 in Roane's garden, R06-18, cited above; appeared on incubated leaves and spikelets. Determined from keys of Boothe (1971) and Nelson et al. (1983). NR, U.

Myrothecium roridum Tode:Fr. – Collected on 2 at the Walton site above, R04-33, Aug. 14, 2004. Characterized by sessile sporodochia subtended by fringes of white hyphae. Conidia measured 8-11 X 3.0-3.5μm (longer than published by Ellis 1971), were cylindrical, hyaline (but black in mass). It has been collected on *Glyceria* and *Poa* (this publication) but not on *Eragrostis* spp. NR, U.

Nigrospora sphaerica (Sacc.) E. Mason – Fruited on incubated spikelets of 4 collected at the Pembroke quarry site above, R06-27, Oct. 8, 2006. This fungus has been found on many grasses in Virginia but not on *Eragrostis* spp. NR, U.

Deuteromy cotina-Coelomy cetes:

Collectorichum caudatum (Sacc.) Peck – Fruited on foliage and spikelets of 4 collected at the Pembroke quarry site above, R06-27, collected Oct. 8, 2006 and on

foliage from the same site, R02-18, collected Sept. 2, 2007. Macroscopic appearance is similar to that of C. graminicola, but conidia terminate in an unbranched, filiform appendage 10-16 $\mu$ m long (Sutton 1980). It is reported on 4 in Oklahoma (Farr et al. 1989). NR, EU.

*Macrophoma phlei* Tehon and G. L. Stout – Collected on foliage of 4 at the Pembroke quarry site above, Oct. 8, 2006, R06-27 and Sept. 2, 2007, R07-18. Pycnidiospores measure 25-27 X 8-11μm, are produced in large dark pycnidia (Sprague 1950). Not reported on *Eragrostis* spp. (Farr et al. 1989). NR, U.

*Phoma sorghina* (Sacc.) Boer., Doren., and VanKest. – Collected on 3 at the Walton site above, R05-28, Aug. 2, 2005. Pycnidiospores measured 5-6 X 2-3μm; illustrated and described (under *Phyllosticta*) by Sprague (1950). Not reported on *Eragrostis* spp. (Farr et al. 1989). Collected on 4 at the Pembroke quarry site, R06-38, Oct. 8, 2006. NR, U.

Stagonospora montagnei Cast. and Germ. = S. graminella (Sacc.) Sacc. – Occurred on foliage of 3 collected at the Walton site above, R05-28. In Sprague's (1950) key the fungus is S. graminella and is considered by him to be definitely saprophytic. Pycnidiospores were pale yellow brown, 1-3-septate, slightly constructed at septa, measuring 15-20 X 3-5µm. Only S. maculata on 2 (R and R 1997, p. 16) has been previously reported from Eragrostis spp. NR, U.

Stagonospora spp. – A fungus resembling S. simplicior was collected on 4 at the Pembroke quarry site Oct. 8, 2006, R06-27. S. simplicior conidia are described as having 3 septa (Sprague, 1950). The R06-27 collection invariably had 4 septa measured 23-28 X 8μm. From appearances it was a 4-septate version of S. simplicior. Each cell was filled withy a single large guttule as in S. simplicior. Whether or not it is S. simplicior, it seems unique and is reported for the first time. NR, U.

# Festuca spp., fescue

- 1. Festuca elatior L. (including F. arundinacea Schreb., and F. pratensis Huds.), Syn. Schedonorus arundinaceus (Schreb.) Dumort, meadow fescue.
- 2. F. obtusa Biehler, nodding fescue.
- 3. F. rubra L., red fescue.
- 4. F. valesiaca Schleich. Ex Gaud., cv. 'Glaucantha'.

# Ascomycotina:

Claviceps purpurea (Fr.:Fr.) Tul. – The ergot fungus is widely distributed on 1 in Virginia but this collection came from 3 at the parking area for Havens Wildlife Management Area, Rt. 622, = Bradshaw Rd., Roanoke Co., El. 1610', N37° 19.840', W80° 9.283', July 25, 2004, R04-27. This is my first collection on 3, but Farr et al. (1989) list it as occurring in eastern states. NR, V.

Phaeosphaeria nigrans (Rob. Ex Desm.) L. Holm. – Collected on 4 at the Community Arboretum, Roanoke, Roanoke Co., Sept. 9, 2004, El. 1090', N37° 14.713', W79° 58.442'. This is an ornamental species, not listed by Farr et al. (1989). Ascospores were 5-septate, 23-24 X 3-4 $\mu$ m, penultimate cell enlarged; illustrated by E and E (1985) and S and B (1989). NR, U.

Basidiomycotina - Uredinales:

Puccinia coronata Cda., II, III., orange crown rust – Although Farr et al. (1989) list this rust as occurring in the range of the host on 1, only one previous collection is recorded in Virginia (R and R 1997). The following simply confirm its broad distribution: Collected by E. L. Stromberg at the Northern Piedmont Agricultural Research Center, Orange Co., Sept. 19, 2006, R06-19; at Dixie Caverns, Roanoke Co., Dow Hollow Rd., El. 1204', N37° 15.51', W80° 10.635', Sept. 9, 2004, R04-50; Roane's garden, 607 Lucas Dr., Blacksburg, Montgomery Co., El. 2175', N37° 14.464, W80° 24.577', Oct. 3, 2004, R04-52.

Deuteromycotina – Hyphomycetes:

Periconia atra Cda. – Occurred on pedicels and spikelets of 1 collected at Walton Montgomery Co., between N.S.R.R. and Rt. 663, creek side, July 20, 2004, R04-24, El. 1740', N37° 9.173', W80° 31.01'. The brown, spherical, verruculose conidia measure 5-9μm (Ellis, 1971). This fungus has been found on several grasses in Virginia (this report); this is the first collection from 1. *P. atra* is not listed by Farr et al. (1989). NR, U.

Spermospora subulata (R. Sprague) Sprague – Collected on **2** at the gate to the FAA Center, White Top Mt., Grayson Co., El. 5520', N36° 38.30', W81° 36.33', June 27, 2004, R04-14. Conidia were hyaline, 1-3-septate, the terminal cell tapering into a whip-like projection, measuring 20-25 X 2-3μm. Prior to 1996, S. subulata had been reported only from the northern Rocky Mts. West to the Pacific (Farr et al. 1989) but has been collected on Arrhenatherum, Bromus, and Danthonia in Virginia (R and R, 1996), and now, Festuca. NR, U.

Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wils. – Numerous associations between C. graminicola and Festuca spp. have been reported (Roane 2004; R and R 1996; 1997), the following notes simply add to host and pathogen range. On 1, Stromberg collected it Sept. 19, 2006 at the Orange Co. site above, R06-19; it was collected on 3 at the White Top Mt. site June 27, 2004, R04-14.

Dinemasporium strigosum (Pers.:Fr.) Sacc., anamorph of Phomatospora dinemasporium J. Webster – Collected on 3 at the Havens Wildlife site above, R04-27, July 25, 2004. It was collected on 4 at the Roanoke Arboretum site above, R04-47, Sept. 9, 2004. Conidiomata are superficial, dark, setose, rounded but becoming cupshaped. Conidia are slightly curved, setulae at both ends, measuring 8-19 X 1.5-2.5  $\mu m$ , illustrated and described by E and E (1985) and Sutton (1980). 3 and 4, NR, U.

*Phaeoseptoria urvilleana* (Speg.) Sprague – Collected on 1 at the Walton, Montgomery Co. site, R04-24, above. This fungus, determined from Sprague's (1943, 1950) keys, is originally from Argentina, but fits perfectly with description by Sprague (1943). Pycnidiospores were 5-7-septate, measuring 30-85 X 4-6μm. It has been found previously on *Elymus, Holcus, Hystrix, Phragmites, Spartina* (R and R 1997), *Agrostis* and *Digitaria* (Roane 2004). Sprague (1950) describes it as saprophytic. Several of the collections above were found above 4000'. NR, U.

*Phoma sorghina* (Sacc.) Boer., Doren., and VanKest. – Collected on foliage of 3 at Havens Wildlife Management Site R04-27 above. Spores measured 4-6 X  $3\mu m$ . The fungus appeared to be associated with leaf spots. NR, U.

Stagonospora avenae (Frank) Bissett – Collected June 20, 2005, R05-7, on **2** at Little Montgomery, Montgomery Co. along Rt. 613 El. 1998', N37° 1.533', W80° 32.475'. Spores were mostly 3-septate, 25-30 X 3-5µm. Fits descriptions and illustrations given by Bissett (1982) and Sprague (1950). NR, U.

#### Glyceria melicaria (Michx.) Hubb., melic-like mannagrass

Myrothecium roridum Tode:Fr. – Collected May 8, 2004 at Little Montgomery, Montgomery Co. site above. On incubated foliage; spores in this collection measured 7-8 X 2μm, were bacillar, smooth, occasionally navicular, with rounded ends. See *Eragrostis*, this publication and Ellis (1971). NR, U.

#### Hakonechloa macra (Munro) Makino, Japanese forest grass

Deuteromycotina – Hyphomycetes:

*Drechslera poae* (Baudys) Shoem. – A cause of eyespots on this grass and *Poa* spp., was collected on Sept. 2, 2004, at the Roanoke Community Arboretum, Western Virginia Community Coll. Roanoke Co., El. 1094', N37° 14.695', W79° 58.455', R04-46. Conidia were light brown, mostly 6-8-septate, measuring 70-90  $\times$  20-28μm. NR, U.

Exserohilum halodes (Drechs.) Leon. and Suggs – Found on leaf sheaths collected at the site above, R04-46, Sept. 9, 2004. Conidia are cylindrical to ellipsoidal, have protruding hila, end cells delimited by dark septa, usually 6-8 septa, measuring 40-90 X 11-19µm. NR, U.

*Nigrospora sphaerica* (Sac.) E. Mason – Appeared on incubated foliage from the Arboretum site above, R04-46. NR, U.

# Holcus lanatus L., velvetgrass

Basidiomycotina - Uredinales:

Puccinia coronata Cda., II, III. – Collected at the "S" curve on Rt. 723, near north fork of Roanoke R. at Ellett, Montgomery Co., June 19, 2007, R07-9, El. 1550' N39° 13.179, W80° 21.883. Apparently, P. coronata is common on every colony of H. lanatus. The alternate host, Rhamnus spp., is rare in Virginia so the fungus may have adapted an autoecious form of life, surviving only as uredinia.

Deuteromy cotina-Coelomy cetes:

Collectorichum graminicola (Ces.) G. W. Wils. – Collected at the gate to the FAA Center, White Top Mt., Grayson Co., June 27, 2004, R04-15, El. 5520', N36° 38.30' W81° 36.331'. This report merely provides more specifics about the White Top Mt. site for collection R94-33 (R and R 1997).

Hystrix patula Moench, Syn., Elymus hystrix L., bottle brush grass

#### Ascomycotina:

Phaeosphaeria eustoma (Fkl.) L. Holm – Collected along road up White Top Mt., Grayson Co., El. 5019', N36° 38.117', W81° 35.314', June 27, 2004, R04-21. Ascospores were 3-septate, measuring 15-24 X 4-5μm. Three fungi are morphologially very similar, *P. eustoma, P, nodorum*, and *P. tritici*. From descriptions by S and B (1989), this fungus fits best into *P. eustoma*. NR, U.

*P. nigrans* (Rob. ex Desm.) L. Holm – Collected on spikelets at the parking area, Falls Ridge, Fagg, Montgomery Co., Oct. 1, 2006, R06-29, El. 1495', N37° 11.523', W80° 31.166'. Ascospores 5-septate, measured 25-30 X 3-4 $\mu$ m. Description fits that by S and B (1989). Previously collected in Virginia (R and R 1997).

#### Deuteromycotina – Hyphomycetes:

Fusarium avenaceum (Fr.:Fr.) Sacc. – Collected on spikelets along Walker Ck., Rt. 708 near Rt. 622, Giles Co., Oct. 22, 2006, R06-35, El. 1634', N37° 17.777', W80° 42.387'. Identified from keys of Boothe (1971) and Nelson et al. (1983). NR, U.

Periconia atra Cda. – Collected on spikelets at the Walker Ck. site above, R06-35. Also collected at the Falls Ridge, Montgomery Co., site above, R06-29. See under *Elymus* and *Festuca*, this publication. NR, U.

*P. lateralis* Ellis and Everh. – Collected on spikelets at the Walker Ck., Giles Co., site above, R06-35. See under *Elymus*, this publication. NR, U.

#### Deuteromycotina – Coelomycetes:

*Phoma sorghina* (Sacc.) Boer., Doren., and Van Kest. – An oft-collected fungus, found on spikelets at the Walker Ck., Giles Co., site, R06-35, and at the Falls Ridge, Montgomery Co., site, R06-29. Spores are oval, 4-5 X 1.5-2.0 $\mu$ m. NR, U.

# Leersia spp., ricegrass

- 1. Leersia oryzoides (L.) Swartz, rice cutgrass.
- 2. L. virginica Willd., whitegrass.

# Ascomycotina:

*Paraphaeosphaeria michotii* (West.) O. Eriks. – Collected on Sept. 20, 2005, on 1, R05-43, at shores of upper pond to the left of entrance, Glen Alton, Giles Co., El. 2627', N37° 25.746', W80° 33.114'; and Oct. 22, 2007 at head of cove between swimming pavilion and superintendent's residence, Claytor L. State Pk., Pulaski Co., El. 1848', N37° 3.203', W80° 37.46', R07-33. Ascospores are dark brown, 2-septate, constricted at septa, rounded ends, measuring 14-18 X 4-5μm. The fungus is described and illustrated by S and B (1985). It was originally described and illustrated by Stout (1930) from collections on maize as *Leptosphaeria zeae*. NR, V.

*Phaeosphaeria eustoma* (Fkl.) L. Holm – Collected Aug. 14, 2005, R05-32, on 2 in field at the junction of Rt. 708 and Forest Service Rd., Montgomery Co., El. 2000', N37° 15.135', W80° 32.011'; and Aug. 29, 2004, on along Rt. 666, Burkes Garden, Tazewell Co., El. 3090', N37° 6.614', W81° 21.516', R04-39. In both collections the fungus appeared to cause leaf spots. Ascospores are 3-septate with enlarged second cell, measuring 15-18 X 4-6μm. Characteristically, there is a single, large globule

filling each cell. This is not mentioned by S and B (1989). For further discussion, see Roane (2004, p. 149). On 1, NR, U.

*P. nigrans* (Rob. and Desm.) L. Holm – Collected on 1 Sept. 20, 2005, at the upper pond, Glen Alton, Giles Co., R05-43 site above, and on **2** Sept. 4, 2005, along the wooded trail south of Pandapas Pond, Montgomery Co., El. 2168', N37° 16.891', W80° 27.924'. Ascospores were 5-septate, second cell enlarged, 15-24 X 3-4μm, associated with bright tan lesions. Farr et al. (1989) report it from Wisconsin and New York. NR, V.

Deuteromycotina – Hyphomycetes:

Bipolaris leersiae (Atk.) Shoem. – Collected on 2 at three locations; at the Forest Service Rd. site above, R05-32, Montgomery Co., Aug. 14, 2005; along the wooded trail of site R05-38, Montgomery Co., at the field where Rt. 703 ends and meets the Forest Service road, Montgomery Co., site R05-32 above, Aug. 14, 2005; and along road below Gatewood Dam, Pulaski Co., El. 2307', N37° 2.376', W80° 51.572', R07-12. Spores were 7-10-septate, measured 50-85 X 10-14μm. The fungus was associated with leaf spots; the Pulaski collection expands its known range for Virginia.

Nigrospora sphaerica (Sacc.) E. Mason – appeared on incubated foliage of 2 collected below Gatewood Dam, site R07-12, above Pulaski Co., Aug. 19, 2007 and at site R05-32 above, Montgomery Co., Aug. 14, 2005. NR, U.

*Tetraploa ellisii* Cke. – Appeared on incubated foliage of 1 collected at the Claytor Lake Park, Pulaski Co., site R07-33 above, Oct. 22, 2007. Conidia had more than 4 cells in their columns. The key by Ellis (1971) distinguishes *T. ellisii* from *T. aristata*; the latter is found more frequently. NR, U.

# Lolium multiflorum Lam., Italian ryegrass

Basidiomycotina – Uredinales:

Puccinia coronata Cda. II, III. – Collected by grower D. Smythers, 163 Fairfield Rd., Woodlawn, Carroll Co., July 23, 2005, R05-23 (Pl. Cl. 05-999). *P. coronata* is widespread on *Lolium* spp. in Virginia but this is the only collection from Carroll Co.

Deuteromycotina – Hyphomycetes:

Drechslera dictyoides (Drechs.) Shoem. – Collected with the same material as R05-23 above. Conidia characterized by tapering from widest first and second basal cell to tip; 5-6–septate, 92-103 X 12-15μm Ellis (1971). Associated with wilted, necrotic leaf tips. NR, V.

#### Miscanthus sinensis and varieties, Eulalia

- 1. Miscanthus sinensis Anderss.
- 2. M. sinensis var. 'purpurescens'
- 3. M. sinensis var. zebrinus Beal, zebragrass

Note: It may not be necessary to list varieties of *M. sinensis* separately but they seem to support different fungi. *M. sinensis* is not listed in Farr et al. (1989) perhaps because no fungi had been listed on it before 1989. Variety *purpurenscens* is listed by Still (1994) but without an author of the trinomial. Variety *zebrinus* is listed in most comprehensive treatments of Poaceae.

Deuteromycotina – Hyphomycetes:

Bipolaris cynodontis (Marig.) Shoem. – Collected on **2** at the Community Arboretum, Virginia Western Comm. Coll., Roanoke Co., Sept. 9, 2004, El. 1088', N37° 14.709', W79° 58.455', R04-46. Conidia 7-10-septate, 49-64 X 12-13 $\mu$ m, ellipsoidal-cylindrical, broadest at middle, tapering to rounded ends (Ellis 1971). Apparently the cause of leaf spots. On 2, NR, U.

Epicoccum nigrum Link – Generally considered as one of the sooty mold saprophytes, and therefore usually ignored but on 1 appeared to be associated with purple-margined leaf spots. A description appears in Ellis' (1971) book under *E. purpurascens*, synonym for *E. nigrum*. Collected on Plant Clinic specimen 04-289 (R04-05), dated May 12, 2004 from Saunders Bros. Nursery, Piney River, Nelson Co. NR, U.

Nigrospora sphaerica (Sacc.) E. Mason – Collected on 1 at Virginia Tech Horticultural Gardens, Blacksburg, Montgomery Co., El. 2166', N37° 13.188', W80° 25.466', Oct. 7, 2007, R07-27. Conidia of this fungus remind me of a ripe olive on a golf tee. NR, U.

Deuteromycotina – Coelomycetes:

Collectorichum graminicola (Ces.) G. W. Wils. – Collected on 3 at the Community Arboretum site above in a different area, El. 1082' N37° 14.699', W79° 58.448', Sept. 9, 2004, R04-45. The white or yellow bands of zebragrass are very much more susceptible to colonization by *C. graminicola* than the green leaf area, to the extent that banded areas on lower leaves are almost universally colonized. On 3, NR, V.

Stagonospora culmicola (Sacc.) Cast. and Germ. – Collected on **3** at the R04-45 site immediately above, Sept. 9, 2005. In old literature, this fungus is assigned to Hendersonia (Sprague 1950). Pycnidiospores were 3-7-, mostly 5-septate, measuring 30-38 X 3-5 $\mu$ m, often accompanying *C. graminicola*. NR, U.

Stagonospora simplicior Sacc. and Briard. – Appeared on incubated leaves of 1 collected at the Va. Tech Horticultural Garden site, El. 2166', N37° 13.188', W80° 25.466', R07-27; associated with leaf spots. This fungus has short, broad, 3-septate spores with large globules in each cell, measuring 24-44 X 9-11 $\mu$ m; they are easily distinguished from other *Stagonospora* spores. NR, U.

# Muhlenbergia spp., muhly

- 1. Muhlenbergia mexicana (L.) Trinius, Mexican muhly, wirestem muhly.
- 2. M. schreberi Gmel., nimblewill.
- 3. M. sylvatica (Torrey) Gray, woodland muhly.
- 4. M. tenuiflora (Willd.) B.S.P., slender-flowered muhly.

# Ascomycotina:

Phyllachora vulgata Theiss and Syd., cause of tar spot – Collected on 2 from three locations: Backyard at 907 Mason Dr., Blacksburg, Montgomery Co., Oct. 6, 2004, R04-54; along road from parking area to Pandapas Pond, Montgomery Co., El. 2216', N37° 16.966', W80° 28.107', Oct. 16, 2005, R05-42; head of cove between swim beach and superintendent's residence, Claytor L. State Pk., Pulaski Co., El. 1848', N37° 3.203', W80° 37.46', Oct. 15, 2007, R07-30. On 3, below Gatewood Dam, Pulaski Co.,

El. 2113', N37° 2.368', W80° 51.573', Aug. 15, 2004, R04-30. *P. vulgata* is described

by Sprague (1950). On 3, NR, V.

Sordaria fimicola (Rob. ex Desm.) Ces. and DeNot. – Typically a dung fungus, also known on Zea; collected on 2 at Wind Rock on Appalachian Trail 1/8 mi. N. of Rt. 613, Giles Co., El. 4125', W37° 24.855', W80° 31.166', Aug. 27, 2006, R06-17. The fungus has black, one-celled ascospores measuring 25-28 X 17-20 $\mu$ m. It is discussed and illustrated in many mycology textbooks. NR, U.

Basidiomycotina - Uredinales:

Puccinia schedonnardi Kell. and Swingle – Collected on 1 from the town park behind 607 Lucas Dr., Blacksburg, Montgomery Co., El. 2175' N37° 14.440', W80° 24.575', Nov. 1, 2004 R04-63; and on 2 at the Mason Dr. site above, Oct. 6, 2004, R04-54. It was previously collected in Virginia on 1 (Roane 2004).

Deuteromycotina – Hyphomycetes:

Bipolaris cynodontis (Marig.) Shoem. – Collected on 1 in the town park area behind 607 Lucas Dr., the site immediately above, Nov. 1, 2004, R04-63; on 2 at the Claytor L. State Pk. site above, Nov. 20, 2004, R04-64, and at the Pandapas Pond site above, Oct. 16, 2005, R05-49; on 3 at the Gatewood Dam site above, Aug. 15, 2004, R04-30; on 4 along Peak Ck. below Gatewood Dam, at N.S.R.R. bridge, Pulaski Co., El. 2110', N37° 1.879', W80° 50.968', R07-14. Conidia were mostly straight, cylindrical or slightly elliptical, widest near middle, 5-8-septate, measuring 40-65 X 5-8μm. On 4, NR, U.

Curvularia brachyspora Boedijn – Appeared on incubated leaves and stems of 2 collected Aug. 27, 2006 at the Wind Rock, Giles Co. site above, R06-17. Conidia were nearly straight, 3-septate, middle septum median, second or third cell may be enlarged, measuring 23-30 X 9-10 $\mu$ m. See Ellis (1971). NR, U.

Drechslera dematioidea (Bubák and Wróbl.) Subr. and Jain – Appeared on incubated leaves of 3 collected along Walker Ck., Rt. 708 near Rt. 622, Giles Co., Oct. 22, 2006, El. 1634', N37° 17.777', W80° 42.387', R06-34. Conidia were yellow-brown, straight or slightly curved, cylindrical to clavate, widest at middle, rounded ends, 3-6-septate, mostly 35-45 X 9-12μm. See Ellis (1971) and Sprague (1950). NR, U.

Exserohilum halodes (Drechs.) Leonard and Suggs – Appeared on incubated leaves and stems of 2 collected Aug. 27, 2006, at the Wind Rock, Giles Co. site above (R06-17). Conidia were yellow-brown, widest near middle, end cells usually delimited by a thick, dark septum, had a protruding hilum, were 7-9-septate, 55-80 X 14-15μm. See Ellis (1971) and Sprague (1950). NR, U.

Nigrospora sphaerica (Sacc.) Mason – Collected on 3 below Gatewood Dam, Pulaski Co. site R04-30 above, Aug. 15, 2004. It was reported on 3 from Montgomery Co. by R and R (1997).

Periconia atra Cda. – Collected on 1, 2, and 3. On 1, in town park behind 607 Lucas Dr., Montgomery Co. site R04-63, Nov. 1, 2004. On 2, at the Pandapas Pond site R05-49, above, Oct. 16, 2005. On 3 at the Walker Ck., Giles Co. site R06-34 above, Oct. 22, 2006. Conidia are spherical, verruculose, 5-9μm, diam. See Ellis (1971) for description. On 1, 2, 3. NR, U.

*Periconia byssoides* Pers. – Collected on 1 at Claytor L. State Pk., Pulaski Co., on point uplake from swim beach, Nov. 20, 2004, R04-64. Conidia are spherical, larger (10-12  $\mu$ m) than for *P. atra* above and have morphologically different conidiophores. See Ellis (1971). NR, V.

Periconia lateralis Ell. and Everh. – Collected on 2 and 3: On 2, at head of cove between swim beach and superintendent's residence Claytor L. State Pk., Pulaski Co., Ot. 15, 2007, R07-30; on 3 at the Walker Ck., Giles Co. site, R06-34 above, Oct. 22, 2006. Conidiophores extend to a tapering point beyond sporulation site. Conidia are spherical, verruculose, 8-15µm diam., Ellis (1971). On 2, NR, V; on 3, NR, U.

#### Deuteromycotina – Coelomycetes:

Ascochyta graminea (Sacc.) Sprague and Johnson – Collected on 1 at the town park site behind 607 Lucas Dr., Blacksburg, Montgomery Co., Nov. 1, 2004. See Sprague (1950) and Punithalingam (1979). It persisted at this site from Nov. 2003 to summer 2007.

Colletotrichum graminicola (Ces.) G. W. Wils. – Collected on **2** on point uplake from swim beach, Claytor L. State Pk., Pulaski Co., Nov. 20, 2004, R04-64.

Stagonospora montagnei Cast. and Germ - = S. graminella Sacc. - Collected on 2 at the Claytor L. State Pk. site immediately above, R04-64. Sprague (1949) provides a description under S. graminella and a comparison with other similar species. Farr et al. (1989) list it on *Muhlenbergia* spp. only from New Mexico. Pycnidiospores measured 20-22 X 3-4  $\mu$ m, were usually constricted at the 3 septa. It was collected on 2 at another site in the park (R and R 1997).

#### Deuteromycotina - Other:

Rhizoctonia solani Kühn, cause of summer blight – Collected on 2 in field next to parking area Aug. 20, 2006, at Falls Ridge, Fagg, Montgomery Co., El. 1455' N37° 11.523', W80° 19.247', R06-07. Summer blight also occurred on plants of *Festuca elatior* close by. Farr et al. (1989) have a long list of hosts for this fungus, including many grasses. On 2, NR, V.

# Panicum spp., panic grass

- 1. Panicum anceps Michx., flat-stemmed panic grass.
- 2. P. virgatum L., switchgrass.

# Deuteromycotina – Hyphomycetes:

*Bipolaris sorokiniana* (Sacc.) Shoem. – Collected Oct. 7, 2007 on **2** at Horticultural Gardens, Virginia Tech, Blacksburg, Montgomery Co., El. 2166', N37° 13.215', W80° 25.458', R07-25. A well-known fungus with a broad graminicolous host range. It was associated with leaf spots; seems to be universally present on **2** (R and R 1997; Roane 2004).

Nigrospora sphaerica (Sacc.) E. Mason. – Collected on 2, Oct. 7, 2007, at the Horticultural Garden site above, R07-25. This fungus has been reported numerous times on grasses in this report, yet is not reported on 2. NR, U.

Deuteromycotina - Coelomycetes:

Amerosporium atrum (Fkl.) Höhn – Collected July 20, 2005, on 1 on Gap Mt. Forest Service Rd., about ½ mi W. of U.S. 460, Montgomery Co., El. 2378', N37° 16.755', W80° 28.971', R05-20. The genus Amerosporium is described by Sutton (1980). A. atrum is characterized in a key to Sphaeropsidales and illustrated by von Arx (1981). NR, U.

Stagonospora simplicior Sacc. and Briard – Collected on 1 during a revisit to site R05-20 above, Aug. 5, 2005, R05-31. It is characterized by easily recognized broad, 3-septate conidia measuring 24-44  $\times$  9-11  $\mu$ m, usually having a single large globule in each cell (Sprague 1950). NR, U.

#### Paspalum spp.

- 1. Paspalum dilatatum Poir., Dallis grass.
- 2. P. laeve Michx., smooth or field paspalum.

#### Ascomycotina:

Claviceps paspali Stevens and Hall, in honeydew or Sphacelia (conidial) stage — Collected on 1, Oct. 15, 2007, at the head of the cove between the swim beach and Supt. residence, Claytor L. State Pk., Pulaski Co., El. 1848', N37° 3.203', W80° 37.460', R07-31. I could find only one paper describing the conidia of the honeydew stage (Brown and Ranck 1915). Stevens and Hall (1910) who described the sclerotia and ascomata did not describe conidia. In this collection, R07-31, conidia were hyaline, 1-celled usually cylindrical, with rounded ends 7-14 X 4-6μm. Brown and Ranck (1915) described them as, "single celled, about 5μ wide and 15μ long, oblong, hyaline, with contents homogeneous except for a cluster of granules at each end". This description fits the Claytor L. State Pk. collection. No name has been applied to this anamorphic stage as it has for C. purpurea, Sphacelia segetum Lév. C. paspali is very common on P. dilatatum but by Oct. 15 it is usually in the sclerotial stage or the host has been killed by frost.

Deuteromycotina – Hyphomycetes:

Curvularia lunata (Wakk.) Boed. – Collected on **2**, Sept. 14, 2007, along the sloping lawn south of the Solitude parking lot, Virginia Tech, Blacksburg, Montgomery Co., El. 2037', N37° 13.560', W80° 25.553', R07-22. The fungus was abundantly present on necrotic blades, Conidia are asymmetrical, end cells light, 3-septate, middle septum not median, measuring 20-32 X 9-15μm (Ellis 1971). On **2**, NR, U.

*Myrothecium atroviride* (Berk. and Br.) Tulloch – Collected on the Solitude lawn site above, R07-22, Sept. 14, 2007. Sporodochia were sessile, conidia olive-brown, measuring 11-13 X 3.0-3.5μm. Illustrated by Ellis (1971). On **2**, NR, U.

# Pennisetum glaucum (L.) R. Br., pearl millet

#### Ascomycotina:

*Gibberella zeae* (Schw.) Petch, the cause of scab and head blight. – Sent to the Plant Clinic (Pl. Cl. 04-1301) Oct. 10, 2004, by Carl Stafford, Co. Agt., collected on Mt. Pony Farm, Culpeper Co., R04-59. Nearly all kernels on several heads were covered by ascomata bearing asci with hyaline, 3-septate ascospores measuring  $18-25 \times 3-4 \mu m$ .

Conidia of the *Fusarium graminearum* anamorph were also prevalent. A 5-acre crop being produced for bird seed was totally destroyed. Farr et al. (1989) do not list *Pennisetum* as a host. NR, U.

Phaeosphaeria tritici (Garev.) Hedj. – Present on the pedicels of the specimen above, R04-59. Ascospores were 3-septate, measured 18-20 X 4-5 $\mu$ m. (See S and B 1989). NR, U.

#### Phalaris arundinacea L., reed canary grass

Two types of *Phalaris arundinacea* were found on White Top Mt., Grayson Co.; the typical green-leaved type and *P. a.* var. *picta* L., ribbon grass, with white-striped leaves.

#### Ascomycotina:

*Phyllachora phalaridis* Orton – Found on *P. arundinacea* opposite a spring on road up White Top Mt., Grayson Co., El. 5025', N36° 38.049', W81° 36.22', June 27, 2004, R04-18. Ascospores measured 9-10 X 4-6 $\mu$ m. For details see Orton (1944) and Sprague (1950). In Farr et al. (1989), *P. phalaridis* is reported only from Mississippi and Massachusetts. NR, V.

#### Deuteromycotina – Coelomycetes:

Septoria bromi Sacc. var. phalaricola Sprague – Collected on same plants as in R04-18 above. Pycnidiospores usually 3-septate, occasionally 4-septate, 30-55 X 2-3µm. Sprague (1950) provides a key to Septoria spp. Farr et al. (1989) list collections only from Washington and Oregon. NR, EU.

Stagonospora foliicola (Bres.) Bubák – Found on same plants in collection R04-18 above and also on *P. a.* var. *picta*, R04-19, at the same White Top Mt. location as above. Pycnidiospores of *S. foliicola* are very different from those of *S. bromi* var. *phalaricola*. The former causes tawny blotch, a widespread disease of *Phalaris*. Spores are blunt at the base, acute at the apex usually constricted at the 6-12 septa, measuring 40-90 X 5-7µm (Sprague 1950). On *P. a.* var. *picta*, NR, V.

# Phleum pratense L., timothy

# Deuteromycotina – Hyphomycetes:

Bipolaris sorokiniama (Sacc.) Shoem. – Although B. sorokiniana has a very wide host range, it has not been found previously on *Phleum* in eastern U.S.A. This specimen, R06-9, was sent to the Plant Clinic (Pl.Cl. 06-886) from Orange Co., July 27, 2006. The 7-10-septate, dark brown conidia are variously shaped but most are elliptical-ovate, measuring 60-100 X 10-20μm. NR, EU.

# Deuteromycotina – Coelomycetes:

Colletotrichum graminicola (Ces.) G. W. Wils. – Collected June 27, 2004, at the gate to the FAA Center, White Top Mt., Grayson Co., El. 5510', N36° 38.30' W81° 36.331', R04-16. Acervuli were prevalent on leaf sheaths.

Stagonospora maculata Cast. and Germ. – Associated with leaf spots collected July 10, 2005, along Rt. 666 W, Burkes Garden, Tazewell Co., El. 3082', N37° 6.418', W81° 21.214', R05-16. This fungus known primarily for its damage to orchardgrass, *Dactylis* 

glomerata, has a relatively small host range. Spores are described by Sprague (1950) as fusiform, rounded ends, constricted at the 3 or 4 septa, measuring 27-40 X 5.0- $6.5\mu m$ . NR, U.

#### Poa spp., bluegrass, speargrass

- 1. Poa alsodes Gray, woodland bluegrass.
- 2. P. compressa L., Canada bluegrass.
- 3. P. pratensis L., Kentucky bluegrass.
- 4. P. sylvestris Gray, sylvan or woodland bluegrass.

#### Ascomycotina:

Paraphaeosphaeria michotii (West.) O. Eriks. – Collected on **2**, Aug. 17, 2004 at the Butt Mt. overlook, Giles Co., El. 4200', N37° 22.137', W80° 37.413', R04-35; on **4**, May 8, 2004, at Little Montgomery, Montgomery Co., near end of Rt. 613, El. 1998', N37° 1.533', W80° 32.475'. The fungus is characterized by brown cylindrical ascospores, constricted at the 2 septa, rounded ends, measuring 14-22 X 3-6μm, described and illustrated by Dennis (1978), and S and B (1985). On **2** and **4**, NR, U.

Phaeosphaeria eustoma (Fkl.) L. Holm – Occurred on spikelets of **2** collected along Taylor's Hollow Rd., Rt. 712, Ellett, Montgomery Co., El. 1505', N37° 12.418', W80° 21.035', Oct. 1, 2006, R06-23. Ascospores are 3-septate, penultimate cell enlarged, measuring 17-29 X 4-5μm (S and B 1989). On **2**, NR, U.

Sordaria fimicola (Rob. ex Desm.) Ces. and DeNot. – Collected on 2, Aug. 27, 2006, on Appalachian Trail 1/8 mi toward Wind Rock from Rt. 613, Giles Co., El. 4125', N37° 24.855', W80° 31.166', R06-14. Ascospores are black, subglobose to elliptical, uniseriate, measuring 19-25 X 10-13μm (Dennis, 1978). It was collected on *Muhlenbergia schreberi* at this same site. It is illustrated in many mycology textbooks. NR, V.

# Basidiomycotina - Uredinales:

Puccinia brachypodii Otth var. poae-nemoralis (Otth) Cummins and H.C. Greene. – Collected on 2 at four locations: On bank of New River, ¼ mi. above Belspring but in Montgomery Co., El. 1656', N37° 11.058', W80° 35.304', June 30, 2004, R04-22; at the Wind Rock site above, Giles Co., Aug. 8, 2006, R06-5; at the Taylor's Hollow Rd. site above, Montgomery Co., Oct. 1, 2006; on Rt. 603, Rocky Gap—Captain Road, Craig Co., El. 2437', N37° 22.671', W80° 26.799', Aug. 26, 2007, R07-15. Collections had stages II and III; the fungus was identified with the aid of keys by Cummins (1971); it is characterized by "cylindric-capitate or capitate, paraphyses, ....usually geniculate and with a constricted neck" (Cummins 1971). The fungus seems to be universally associated with 2 in southwestern Virginia.

Puccinia recondita Rob. ex Desm., leaf rust – Collected on 2 at Montgomery Tunnels, Montgomery Co., El. 1815', N37° 9.466', W80° 19.175', June 11, 2004, R04-12. Widespread on 3.

# Deuteromycotina - Hyphomycetes:

Bipolaris sorokiniana (Sacc.) Shoem. – Collected on 4 June 20, 2005 at Little Montgomery, Rt. 613, Montgomery Co. El. 1998', N37° 1.533', W80° 32.475', R05-10.

A widespread fungus on grass hosts. This specimen sporulated at the nodes; appeared to be causing a nodal rot. On 4, NR, U.

Curvularia inaequalis (Shear) Boedijn – Sporulated on incubated pedicels and spikelets collected on **2** at the Wind Rock, Giles Co. site above, Aug. 27, 2006, R06-14. Conidia are straight or slightly curved, 4-septate, center cell largest, end cells lighter colored, measuring 27-34 X 9-11µm. NR, U.

Curvularia lunata (Wakk.) Boedijn – Collected on 4 at the Little Montgomery site above on Rt. 613, Montgomery Co., May 8, 2004, R04-4, Conidia were curved, 3-septate, penultimate cell enlarged, end cells hyaline, measuring 22-30 X 9-11μm (Ellis 1971). NR, U.

*Myrothecium gramineum* Lib. – Collected on **3** at the Montgomery Tunnels site above, June 11, 2004, R04-12. Setose sporodochia bear pale olivaceous conidia measuring 7-10 X 2μm (Ellis 1971). On **3**, NR, V.

Myrothecium roridum Tode:Fr. – A specimen of 3 sent to the Plant Clinic (Pl.Cl. 05-1027) from a grower in Albemarle Co., July 28, 2005, who complained that something on the grass was making horses ill. We could not attribute the problem to any fungus. The sporodochia of M. roridum lack setae, conidia were pale olive, cylindrical, with rounded ends, measuring 6-8 X 1.5-2.5µm (Ellis 1971). NR, U.

*Nigrospora sphaerica* (Sacc.) E. Mason – Collected on **2** at the Butt Mt., Giles Co. site above, Aug. 17, 2004, R04-35; described and illustrated by Ellis (1971). On 2, NR, U.

Periconia byssoides Per. ex Merat – Collected June 11, 2004, on 3 at the Montgomery Tunnels, Montgomery Co. site above, R04-12. Fungus appeared on incubated foliage and inflorescence parts. Conidia were spherical, brown, verrucose, measuring 10-15 $\mu$ m dia. (Ellis 1971). On 3, NR, U.

Deuteromycotina – Coelomycetes:

Ascochyta sorghi Sacc. – Collected on three Poa spp.: On 1 on bank of New R., ¼ mi. above Belspring, Montgomery Co. side, June 14, 2005, R05-6, El. 1656', N37° 11.058', W80° 35.304', apparently causing blade necrosis; previously collected on 1 (Roane, 2004). Collected on 2 at New R. site above June 30, 2004, R04-22. Possibly causing leaf tip necrosis. Collected previously in Montgomery Co. (R and R 1997). Collected on 3 at the Montgomery Tunnels site June 11, 2004, R04-12. See Sprague (1950) for description and illustration. On 3, NR, U.

Ascohyta subalpina Sprague and Johnson – Collected on nodes of 4 at the Little Montgomery site above R05-10, June 20, 2005. Spores were 1-septate, bacillar, measuring 11-14 X 2μm. Both Sprague (1950) and Punithalingham (1979) devote considerable discussion to distinguishing this species. In keys of Sprague, it is difficult to distinguish it from Septoria oudemansii (see below). This material seems to fit best A. subalpina. NR, U.

Colletotrichum graminicola (Ces.) G. W. Wils. – Collected on three species: On 2 the New R. bank site above, R04-22. Fruited on leaf sheaths. On 3 at Ironto, east of N.S.R.R. M.P. 266, Montgomery Co. El. 1298', N37° 13.47', W80° 16.334', June 10, 2007. On 4 at Little Montgomery, Montgomery Co., along Rt. 613, site R04-4 above, May 8, 2004. On 4, NR, U. On 2 NR, V.

Coniothyrium scirpi Trail – Collected on spikelets of **2** at the Butt Mt., Giles Co. site above, Aug. 17, 2004, R04-35. This fungus is the anamorph of *Paraphaeosphaeria* 

*michotii* (see above) which was present on leaves of **2** of this collection. For further discussion of the anamorph-teleomorph relations, see *C. zeae* in Sprague (1950), *P. michotii* in S and B (1985), and *Leptosphaeria michotii* in Dennis (1978). On 2, NR, U.

Dinemasporium strigosum (Pers. ex Fr.) Sacc. – Collected on 2 July 8, 2006 along Appalachian Trail, at Wind Rock, 1/8 mi. N. of Rt. 613, Giles Co., site R06-5 above, El. 4125', N37° 24.855', N80° 31.166'. This fungus is described and illustrated by Sutton (1980). On 2, NR, U.

Septoria oudemansii Sacc. – Fruited on leaves and florets of 2 collected Oct. 1, 2006, along Taylor's Hollow Rd., Rt. 712, Ellett, Montgomery Co., El. 1505', N37° 12.418', W80° 21.035', R06-23; on 2 collected Aug. 8, 2006, at the Wind Rock, Giles Co. site R06-5 above; and on spikelets of 2 collected Aug. 27, 2006 at the Wind Rock site, R06-14 above. This fungus has conidia more like those of *Ascochyta*. In the collections above they measured 14-17 X 2 $\mu$ m, were widest at the single median septum. See Sprague (1950) for further discussion. On 2, NR, V.

Schizachyrium scoparium (Michx.) Nash, little bluestem – See Andropogon scoparius.

#### Secale cereale L., rye

Basidiomycotina – Uredinales:

*Puccinia recondita* Rob. ex Desm., II, III – Collected June 12, 2008 at Little Montgomery, Montgomery Co. along Rt. 613, El. 1998', N37° 1.533' W80° 32.475', R05-5. This is a common leaf rust of cereals and grasses. It occurs on rye throughout Virginia.

Deuteromycotina – Hyphomycetes:

Bipolaris sorokiniana (Sacc.) Shoem. – A common fungus on cereals in Virginia, it has not been collected on rye before. It was collected on rye growing from straw mulch at the Little Montgomery site above, R05-5. NR, V.

Setaria faberi Herrm., giant foxtail

All fungi on this host were collected at the parking area between Rt. 663 and the N.S.R.R., Walton, Montgomery Co., El. 1824', 37° 9.176', W80° 31.007' on Aug. 2, 2005, R05-30.

Deuteromycotina – Hyphomycetes:

*Drechslera erythrospila* (Drechs.) Shoem. Associated with leaf spots. Conidia measured 62-65 X 11-15 $\mu$ m, were 8-10-septate, cylindrical, with rounded ends. See Ellis (1971) for description and illustrations. NR, U.

*Pyricularia grisea* (Cooke) Sacc. – The cause of gray leaf spot of several grasses, this fungus has pear-shaped, 2-septate conidia, sometimes with protruding hilum, measuring 17-28 X 6-9 $\mu$ m. See Ellis (1971) for illustration and description and R and R (1996) and Roane (2004) for additional distribution.

Deuteromycotina - Coelomycetes:

*Phoma sorghina* (Sacc.) Boer., Doren., and VanKest. – Pycnidiospores measured 6-7 X 2-3μm. It is widely distributed in Virginia. NR, U.

Sorghastrum nutans (L.) Nash, Indian grass

Deuteromycotina – Coelomycetes:

Stagonospora simplicior Sacc. and Briard – Collected Sept. 16, 2007 at old garden area, Glen Alton, Giles Co., near Big Stony Ck., El. 2606', N37° 25.860', W80° 33.004', R07-23. The broad 3-septate, globulate spores measuring 24-44 X 9-11µm are readily distinguished from other *Stagonospora* spp. (Sprague 1950). Causes a leaf spot common on *S. nutans*.

Sorghum bicolor (L.) Moench, grain sorghum, broom corn, sweet sorghum, etc.

Deuteromycotina – Hyphomycetes:

Gloeocercospora sorghi Bain and Edgerton – Causing seedling leaf spot on Pl. Clinic specimen 07-610, R07-8, received June 18, 2007 from Allen Straw, S. W. Agri. Res. and Ext. Ctr., Glade Spring for grower Bill Sally of Scott Co. On adult plants, causes zonate leaf spot. Spores are described by Sprague (1950); a complete description and illustrations are in the Compedium of Sorghum Diseases (Frederiksen, 1986). NR, V.

Stenotaphrum secundatum (Walt.) Kuntze, St. Augustine grass

Ascomycotina:

Gaeumannomyces graminis (Sacc.) von Arx and D. Olivier, cause of take-all – Plant Clinic specimen sent in by J. Orband, Ext. Agt., and Joan Ward, grower from Hampton, York Co., Aug. 22, 2006, Pl. Cl. 06-1049, R06-20. Runner hyphae and hyphopodia were clearly evident and are diagnostic. NR, V.

Sphenopholis intermedia (Rydb.) Rydb., slender wedgegrass

Note: This species has also been named *S. intermedia* var. o*btusata* (Torrey) K. S. Erdman. (Gould and Shaw 1983). T. F. Wieboldt of the Massey Herbarium at Virginia Tech identified this specimen.

Basidiomycotina – Uredinales:

*Puccinia eatoniae* Arth.; II, III – Collected at Little Montgomery, Montgomery Co. on Rt. 613, ¼ mi. from junction with Rt. 620, June 12, 2005, El. 1998', N37° 1.533', W80° 30.475', R05-4. The taxonomy of *S. intermedia* has been revised since the publication by Farr et al. (1989), this collection is considered a NR, V.

Tridens flavus (L.) Hitchcock, purpletop, grease grass

Ascomycotina:

*Phaeosphaeria eustoma* (Fkl.) L. Holm – Collected at head of the cove between the swim beach and superintendent's residence, Claytor L. State Pk., Pulaski Co., Oct. 22,

2007, El. 1848', N37° 3.203', W80° 37.460', R07-32. Ascospores were 3-septate, constricted at septa, measured 17-29 X 4-6μm, penultimate cell enlarged, fruiting on leaf sheaths. It was identified from keys in E and E (1985); it has been collected in another part of the Park (R and R 1997).

Basidiomycotina – Uredinales:

*Puccinia windsoriae* Schw., II, III – Collected at the United Methodist Church area near intersection of Rts. 603 and 713, Montgomery Co. Oct. 17, 2004, R04-58; and at the Claytor L. State Pk. site above, R07-32, Oct. 22, 2007. Stages and II, III present. This rust apparently is ubiquitous on *T. flavus* in Virginia (Roane 2004; R and R 1997).

Deuteromycotina – Hyphomycetes:

Curvularia clavata Jain – Collected at the Claytor L. State Pk. site above, R07-32, Oct. 22, 2007. The mostly straight 3-septate conidia, are widest at penultimate cell, middle cells darker than end cells, measuring 25-27 X 8-9μm. Determined from keys by Ellis (1971). NR, U.

Curvularia protuberata Nelson and Hodges – Collected at a field on NW corner of Clay and Jefferson Sts., Blacksburg, Montgomery Co., Oct. 8, 2004, El. 2170', N37° 13.970', W80° 24.202', R04-56. With 4 septa, protruding hila, and being relatively short, 35-42 X 80-12μm, this fungus easily keys to *C. protuberata* (Ellis 1971). This collection widens the range of this association in Virginia.

Deuteromycotina - Coelomycetes:

Neottiosporina paspali (Atk.) Sutton and Alcorn – Initially described as Stagonospora paspali (Sprague 1950), Neottiosporina was separated because the gelatinous sheath surrounding young conidia may adhere to terminal or basal cells as appendages. In this collection, no appendages were observed. However, the 2 septa separate the cells uniformly; conidia were hyaline, cylindrical, sometimes constricted at septa, ends rounded, many with a large vesicle in each cell, measuring 20-24 X 7-8µm. The source of the report of its previous occurrence in Virginia is obscure (Farr et al. 1989; Sprague 1950).

Rhynchosporina tridentis Sprague and Rogerson – The cause of a leaf spot and by virtue of its prevalence, eventual wilting of the leaves. Collected at the Blacksburg site above R04-56, Oct. 5, 2004; and in a field adjacent to the N.S.R.R. crossing of Rt. 603, Ellett, Montgomery Co., Aug. 20, 2006, El. 1462', N37° 11.219', W80° 21.019', R06-6. Like *Puccinia windsoriae*, it is ubiquitous in Virginia on *T. flavus* (R and R 1997).

Stagonospora montagnei Cast. and Germ. [= S. graminella (Sacc.) Sacc.] – Fruited in some of the summer blight lesions (see *Rhizoctonia solani* below), collected Aug. 20, 2006 at Rt. 603 and N.S.R.R., site R06-6 above. Pycnidiospores were straight or curved, cylindrical 3-septate, sometimes constricted at septa, measuring 23-28 X 2-4µm. The fungus was determined from a key by Sprague (1950). NR, U.

Stagonospora simplicior Sacc. and Briard – Associated with oval leaf spots with brown margins, bleached centers, collected at the Claytor L. State Pk. site above, R07-32, Oct. 22, 2007. The broad, 24-44  $\times$  9-11 $\mu$ m, 3-septate pycnidiospores, with large vacuoles, are easily recognized. NR, U.

Deuteromycotina - Other:

Rhizoctonia solani Kühn, cause of summer blight, sharp eyespot, and brown patch of grasses – Collected Aug. 20, 2006, at the Rt. 603, N.S.R.R. crossing area, R06-6, cited above. Although the disease is called spring blight it is common on many grasses well into autumn. No spores are produced but it has mycelial characteristics that make it easily identifiable (Couch 1995, p. 61). NR, V.

#### Triticum aestivum L., wheat

Deuteromycotina – Hyphomycetes:

Hymenula cerealis Ellis and Everh. (= Cephalosporium gramineum Nisikado and Ikata) – Collected at Wildwood Pk., Radford, Montgomery Co., June 10, 2005, El. 2220', N37° 7.855', W80° 34.003', R05-3. Several volunteer wheat plants grew where wheat straw was used to mulch a seeding of ryegrass beside a new walkway. It had been found in volunteer rye where rye straw was used to mulch a new seeding (R and R 1994). Collected in a commercial field at Riner, Montgomery Co., El. 2115', N37° 3.062', W80° 25.511', R06-4, 'Cephalosporium stripe' had never before been found in a commercial wheat field in Virginia, but had been found in two locations used for experimental plants (R and R 1994; Schmale et al. 2007).

#### Zea mays L., maize, corn

Basidiomycotina - Uredinales:

*Puccinia sorghi* Schwein. II, III – corn or maize rust – Collected in Roane's garden, 607 Lucas Dr., Blacksburg, Montgomery Co., Sept. 2, 2004, R04-41, El. 2165', N37° 14.464', W80° 24.577'. *P. sorghi* is widespread on maize in Virginia; this collection was made because many uredinea were colonized by *Sphaerellopis filum* (Biv.- Bern. ex Fr.) Sutton, a hyperparasite of rust fungi (see below).

Deuteromycotina – Hyphomycetes:

Bipolaris maydis (Nisikado and Miyake) Shoem., cause of southern corn leaf blight – Collected from sweet corn by grower T. Baker, Virginia Beach, Aug. 18, 2004, R04-38 (Pl. Cl. 04-1012). Southern corn leaf blight was once a devastating disease in southeastern Virginia but now it is controlled by resistant hybrid varieties. A history of this disease is under its teleomorph, Cochliobolus heterostrophus (R and R 1994).

Deuteromycotina – Coelomycetes:

Collectorichum graminicola (Ces.) G. W. Wils., causing anthracnose – This collection was on sweet corn grown by J. Bates, Amelia Co., sent to the Plant Disease Clinic (Pl. Cl. 04-1202, R04-51) Sept. 21, 2004. Under some conditions anthracnose may be very damaging to field or sweet corn in Virginia.

*Phoma sorghina* (Sacc.) Boer., Doren. and VanKest. occurred on the sweet corn collection above. It was also found in the collection from Virginia Beach site above, R04-38. In this collection only *B. maydis* caused damage. It was collected on corn at Warsaw, Richmond Co. in 1951 under its old name, *Phyllosticta sorghina* Sacc. (R and R 1994).

Sphaerellopsis filum (Biv.-Bern. ex Fr.) Sutton – This hyperparasite of rust fungi occurred in the uredinial sori of *Puccinia sorghi* above, R04-38, from Roane's garden. It has occurred in several rusts collected in Virginia but has not been reported in *P. sorghi*. Spores are 1-septate, measure 17-20 X 5-6μm. Sutton (1980) describes and illustrates the fungus; he lists 27 synonyms for *S. filum*. NR, U.

Stagonospora arenaria Sacc. – occurred on the sweet corn collection from Amelia Co., R04-51, cited above. No Stagonospora spp. are listed on corn by Farr et al. (1989). Pycnidiospores for this collection were cylindrical, 3-4-septate, uniformly 33-35 X 3-4μm. Stagonospora spp. are distributed widely on hosts; it is surprising that this should be the first on corn. Unfortunately, the grower did not furnish enough material for a voucher specimen. NR, U.

#### DISCUSSION

Many new fungus-host associations are reported but no new species of fungi are recognized. Apparently, most fungi in these new associations have a broader host range than previously reported. These new associations have been recognized primarily because the effort to find them is a pioneer enterprise for Virginia. Very few reports have been issued from Virginia since Sprague's (1950) book (Farr et al. 1989). Most Virginia reports cited in his book were issued when the U.S.D.A. Bureau of Plant Industry prior to 1940 was at Arlington Farm, site of the Pentagon. There, numerous grasses were grown, many were plant introductions, to evaluate or enhance their usefulness for forage, turf, and soil conservation. They were scrutinized for plant disease fungi and new findings were promptly reported. Such activity has ceased or greatly declined or most fungus host associations on grasses examined have already been reported. The great number of new associations reported by Roane (2004), R and R (1996, 1997) and the current paper can be attributed to the remoteness of collections and the lack of curiosity of competent mycologists or plant pathologists.

There must be many more new associations awaiting discovery. The great majority of collections reported here have come from Montgomery and the surrounding counties, Craig, Roanoke, Floyd, Pulaski, and Giles, at little more than one-seventeenth of Virginia's 100 original counties. Counties in the Coastal Plain and Piedmont are poorly represented as are those of the northern mountains and the far Southwest. Thus, most of Virginia remains a frontier for fungus-host exploration. Another factor is the rarety of some grass species. Upon examining the distribution maps in the Atlas of the Virginia Flora (Harvill et al. 1992) one can see that many species are known in very few counties, several only on Eastern Shore, and several only in southeastern counties. Roane (1991) describes several as very rare. These remain unexamined. Another factor is sample size. Most reports herein were generated by examining a few leaves, stems, or inflorescenses of a particular species, only an infinitesimal portion of perhaps billions of plants; ex, Agrostis alba, Andropogon virginicus, Cynodon dactylon, Dactylis glomerata, Digitaria sanguinalis, etc. How many fungi are overlooked?

One question that spurred interest in this project, do wild grasses harbor disease – causing fungi of cereal, turf, and forage crops? *Puccinia recondita*, a pathogen of wheat, was found on a number of grasses. However, rust fungi are notoriously specialized in their host ranges, a situation that is well documented in plant pathology literature. *Colletotrichum graminicola*, the anthracnose fungus, was found on the most host species. This ubiquitous fungus is usually saprophytic, but a field of oats in

Pulaski Co. was completely destroyed by it (R and R 1994). It wreaked havoc to corn on Northern Neck for several years, yet in most encounters it seems to be a saprophyte starting early decomposition of its host. Several fungi in the genera *Bipolaris* and *Drechslera* that cause serious diseases of economic plants were found on wild hosts. It is not known whether these fungi could colonize cultivated species; that could be determined only by tedious fungus isolations from various hosts and subsequent inoculation of economic grasses. From the current studies and our other reports (Roane 2004; R and R 1996, 1997), only host-fungus associations have been observed or broadened, not pathogenic capabilities.

The innocuous practice of using cereal straw for mulching new lawn seedings may have undesirable consequenes. On three occasions, cereal plants growing from seed in the straw mulch were observed to have symptoms of Cephalosporium stripe. Twice, rye growing from seed in rye straw was observed displaying stripe symptoms (Jones et al. 1980). Once, wheat straw mulch produced striped wheat plants (Roane, see *Triticum*, this publ.). None of these incidences were in sites that threatened commerical crops; however, it is an established fact that distributing straw from fields with plants infected by *Hymenula cerealis* is a means of further disseminating this destructive fungus.

The reporting of fungus-grass host associations is a contribution to the natural history of Virginia. Since these surveys have covered a relatively small portion of Virginia and many species have not been examined, there remains an opportunity for further discovery. The surprising, sometimes fantastic morphology of microfungi can in itself be an infectious stimulant.

#### **ACKNOWLEDGMENTS**

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# ABSTRACTS OF PAPERS, 87th Annual Meeting of the Virginia Academy of Science, May 27-29, 2009, Virginia Commonwealth University, Richmond VA

# **Aeronautical and Aerospace Sciences**

FROM THE EARTH TO SPACE WITH NACA/NASA. M. Leroy Spearman. NASA-Langley Research Center, Hampton, VA 23681 & Heidi Owens, Auburn University, Auburn, AL 36849. Leonardo da Vinci envisioned man-flight in the 15th century and designed a practical airplane concept in 1490. Many other pioneers proposed various types of flying machines over the next 400 years but it was not until December 17, 1903 that the Wright Brothers, at Kitty Hawk, NC, were credited with achieving the first manned-powered flight. Over the next 100 years, several factors have influenced advances in aviation. The use of aircraft by European nations in World War I resulted in concern that the U.S. was lagging in aviation developments. This lead to an act of the U.S. Congress in 1915 that established the National Advisory Committee for Aeronautics (NACA) with the charge to conduct aerodynamic research. The research began at Langley Field, VA in the early 1920's. Over the years this research has transformed low-speed, wood and fabric, propeller-driven airplanes into high speed, allmetal, jet-propelled airplanes. Jet and rocket propulsion enhanced the fields of supersonic and hypersonic aerodynamic flight and provided for access to space. In July 1955 the White House announced plans to launch an earth-orbiting satellite. Before this was done, however, the Soviet Union successfully launched Sputnik, the world's first artificial satellite in October 1957. This event caused concern that the U.S was lagging in the 'space race' and lead directly to the establishment of the National Aeronautics and Space Administration (NASA) in July 1958. The nucleus of the NASA was the existing NACA with the charge expanded to include space research. The skilled researchers at NASA-Langley have continued to provide improvements in aircraft developments and now contribute to the development of spacecraft as well. Continued advances in aerospace research require well trained researchers. To this end, NASA-Langley participates in mentorship programs to encourage high school students to become researchers. The first author of this paper has been a mentor for many years and the second author of this paper has been a student in the program. Encouragement for researchers is also provided by the VAS and the VJAS.

SIGNIFICANT AERODYNAMIC RESEARCH AT NACA/NASA DURING THE FIRST CENTURY OF FLIGHT. M. Leroy Spearman .NASA- Langley Research Center Hampton, VA, 23681 & Heidi Owens, Auburn University, Auburn, AL 36849. The Wright Brothers are credited with having flown the first manned, heavier-than-air, powered aircraft in December 1903 but the U.S. was slow in accepting the newly introduced airplane. In England, Geoffrey deHavilland produced his first aircraft in1908. In France, Louis Bleriot produced an aircraft in1908. Pre-World War I activities in Europe created concern that the U.S. was lagging behind in the development of aircraft. This concern lead to the creation in the U.S. in 1915 of the National Advisory Committee for Aeronautics (NACA) - a government-funded

research organization that was charged, "to supervise and direct the scientific study of the problems of flight with a view toward their practical solution." Research began at the NACA in the early1920's and has lead to the advancement from low-speed subsonic flight to high-speed transonic, supersonic, and hypersonic flight and to the possibility of achieving space flight. The possibilities of space flight lead to the creation of the National Aeronautics and Space Administration (NASA) in 1958. The NASA absorbed the existing NACA and the charge for aeronautical research was expanded to include space research. Many aerodynamic problems have been analyzed and corrected through wind tunnel testing. In addition to the wind tunnel research, significant aerodynamic results have been provided with rocket-launched pilot-less aircraft as well as the X series of manned aircraft The research conducted by the NACA / NASA has had a direct impact on the design of aircraft and spacecraft for both civil and military systems.

SOME THOUGHTS ON THE HISTORY OF FLIGHT. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA 23681 & Robert W. Heath, RRMC, Newport News, VA. Leonardo da Vinci envisioned man-flight in the 15th century and designed a practical airplane concept in 1490. Many other pioneers proposed various types of flying machines over the next 400 years but it was not until December 17, 1903 that the Wright Brothers, at Kitty Hawk, NC, were credited with achieving the first mannedpowered flight. Over the next 100 years, several factors have influenced advances in aviation. The use of aircraft by European nations in World War I resulted in concern that the U.S. was lagging in aviation developments. This lead to an act of the U.S. Congress in 1915 that established the National Advisory Committee for Aeronautics (NACA) with the charge to conduct aerodynamic research. The research began at Langley Field, VA in the early 1920's. Over the years this research has transformed low-speed, wood and fabric, propeller-driven airplanes into high speed, all-metal, jetpropelled airplanes. Jet and rocket propulsion enhanced the fields of supersonic and hypersonic aerodynamic flight and provided for access to space. In July 1955 the White House announced plans to launch an earth-orbiting satellite. Before this was done, however, the Soviet Union successfully launched Sputnik, the world's first artificial satellite in October 1957. This event caused concern that the U.S was lagging in the 'space race' and lead directly to the establishment of the National Aeronautics and Space Administration (NASA) in July 1958. The nucleus of the NASA was the existing NACA with the charge expanded to include space research. The skilled researchers at NASA-Langley have continued to provide improvements in aircraft developments and now contribute to the development of spacecraft as well.

AN AIRCRAFT DESIGN CONCEPT APPLICABLE FOR VARIOUS MISSION REQUIREMENTS M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA and Katie Klein, MITRE Corp., McLean, VA. Airlift capability can be useful as a means of providing the logistic support of manpower, supplies and equipment in the event of natural disasters such as floods and hurricanes. The need for such support could be within the homeland area or might be at distant worldwide locations. Often, the location for such support may be inaccessible by normal means of transportation. Conventional aircraft can provide the need for speed but the load capacity may be

limited and the requirement for a suitable landing area is critical. An effort to combine the requirements for capacity, speed and basing, has lead to some studies of unconventional aircraft designs. One design concept that has been considered utilizes a large rectangular wing surface with large bodies attached to each wing tip. The use of the two large bodies results in essentially doubling the capacity of a conventional single-body aircraft with no increase in length. The large area of the wing provides adequate lift to sustain normal flight with heavy loads. The bodies could also be shaped to provide for water-based operation. With the wing positioned high on the bodies a cushion of air would be provided that would permit operation as a wing-inground (WIG) effect vehicle. With judicious positioning of trailing-edge wing flaps and vectoring jet nozzles, vertical take-off and landing (VTOL) capability could be achieved. In addition, the bodies could be designed to contain some helium for buoyant lift with additional kinetic lift provided by the wing. Thus, the inboard wing, twin-body arrangement potentially provides for large load carrying capability with a vehicle that could operate in free-air as an airplane, or near the surface in a WIG mode. Such a design would also have greater basing freedom in a VTOL mode or as a hybrid airship.

# **Agriculture, Forestry and Aquaculture Science**

THE EFFECTS OF SHEEP ON NITROGEN CONCENTRATIONS IN SOIL. Sarah J, Casey, Dept. of Biol., Ferrum College, Ferrum, VA 24088 & Brian D. Whitaker, Dept. of Agriculture, Ferrum College, Ferrum, VA 24088. Ruminants are an important part of agriculture because they add value to the existing ecosystem. This study was conducted to evaluate the effects of grazing sheep on agroforestry pasture on the nitrogen content of the soil. Sheep were placed on a traditional grazing pasture or an agroforestry pasture (with trees). Soil samples were collected at 0, 30, and 60 d during the study and analyzed for total nitrogen content at the end of the study. The amount of nitrogen in the soil from the forest without sheep was significantly greater (P < 0.05) compared to the other plots. These results indicate that producing sheep on agroforestry based pasture may increase the quality of the soil by increasing the nitrogen content over time.

NODULATION TRAITS OF TEPARY BEAN INOCULATED WITH 15 BRADYRHIZOBIAL STRAINS. Michele Mohrmann & Harbans L. Bhardwaj, Agricultural Research Station, PO Box 9061, Virginia State University, Petersburg VA 23806. In order to develop tepary bean (Phaseolus acutifolius A. Gray), a highly drought-tolerant summer crop, as a summer legume cover crop to meet N needs of succeeding winter cereals, we studied nodulation following seed treatment of three tepary bean lines (Black, Tan, and White-seeded) with 15 bradyrhizobial strains. In this replicated greenhouse study, we nodule number, and nodule size from approximately 40-day old plants. Nodule numbers were recorded on a scale of 1 (less than five nodules per plant) to 3 (greater than 20 nodules per plant) whereas nodule size was recorded on a scale of 1 (nodules small and similar to mustard/canola seed in size) to 3 (nodules large and similar to soybean seed in size). We also recorded chlorophyll readings with Minolta SPAD-502 meter. Significant differences were observed among

15 bradyrhizobial strains for all traits under study. Differences among three tepary bean lines were not significant. Results indicated that UMR-3007, UMR-3043, and USDA-3254 strains were the most efficient nodulators of tepary bean. Significant and positive correlations exited between SPAD readings and nodule number score (0.70\*\*) and nodule size score (0.43\*\*).

THE ANORECTIC EFFECT OF NEUROPEPTIDE AF IS ASSOCIATED WITH SATIETY-RELATED HYPOTHALAMIC NUCLEI. B.A. Newmyer, M.A. Cline & M. Smith, Radford University, Department of Biology, Radford VA 24142. Neuropeptide AF (NPAF), a member of the RFamide family, is encoded by the same gene as neuropeptide FF (NPFF) which causes short-term anorexia. However, reports role of NPAF on appetite-related process are lacking. intracerebroventricular (i.c.v.) injections of 4.0, 8.0 and 16.0 nmol NPAF were administered to chicks in order to observe its effect on food and water intake. Chicks treated with 8.0 and 16.0 nmol i.c.v. NPAF decreased both their food and water intake. Additionally, all doses of NPAF injected caused a similar reduction in whole blood glucose concentration 180 min after injection. In a second experiment, chicks that received i.c.v. NPAF had increased number of c-Fos immunoreactive cells in the dorsomedial, paraventricular (magnocellular and parvicellular parts) and ventromedial nuclei. The arcuate nucleus and lateral hypothalamic area were not affected. In a third experiment, NPAF-treated chicks exhibited fewer feeding pecks and spent less time perching, while increasing time spent in deep rest. Other behaviours including exploratory pecking, escape attempts, defecations, distance moved, and time spent standing, sitting and preening were not affected by NPAF injection. We conclude that NPAF causes anorectic effects that are associated with the hypothalamus.

CALCITONIN **GENE-RELATED** PEPTIDE IS ASSOCIATED ANOREXIGENIC EFFECTS IN CHICKS (Gallus gallus). Wendy A. Calchary & Mark A. Cline, Department of Biology, Radford University, Radford VA 24142. Calcitonin gene-related peptide (CGRP) is released from the gastrointestinal tract following ingestion and causes satiety in mammals. Its effects on appetite in nonmammalian vertebrates are unreported. In Experiment 1, fasted chicks reduced food and water intake after central injection of CGRP. In Experiment 2, central CGRP caused increased c-Fos immunoreactivity in the arcuate (ARC) nucleus, paraventricular nucleus (PVN), periventricular (PHN) and ventromedial (VMH) hypothalamic nuclei. The results of Experiment 3 demonstrate that intraperitoneal injection of CGRP also causes reduced food and water intake. c-Fos immunoreactivity was increased in the ARC, PHN, PVN and VMH in Experiment 4 after intraperitoneal injection of CGRP. In chicks and mammals stimulation of opioid receptors stimulates feeding. Interestingly, increased CGRP concentration coincides with decrease morphine function in the rodent central nervous system. In Experiment 5, co-injection of CGRP and beta-funaltrexamine did not suppress food intake more than CGRP and betafunaltrexamine when injected alone. In Experiment 6 co-injection of CGRP and ICI-174,864 caused a greater reduction in food intake than CGRP and ICI-174,864 when injected alone. In Experiment 7, co-injection of CGRP and nor-binaltorphimine caused a greater reduction in food intake than CGRP and nor-binaltorphimine when injected

alone. In Experiment 8, CGRP did not reverse hyperphagia induced by NPY. In Experiment 9, hyperphagia induced by B-endorphin was reversed by CGRP. In conclusion, the mechanisms of CGRP induced satiety have some similarities and differences between avian and rodent models. The results presented here provide new insight into the evolution of vertebrate satiety regulatory mechanisms.

BIOLOGICAL NITROGEN FIXATION IN WHITE LUPIN. Harbans L. Bhardwaj, Agricultural Research Station, PO Box 9061, Virginia State University, Petersburg VA 23806. White lupin (Lupinus albus L.), one of five cultivated species of Lupinus genus, has tremendous potential as a grain and a green manuring crop. During 1940s, it was used to supply N to succeeding cotton crop in the southern USA sometimes called "The Lupin Belt". Availability of cheap fertilizers, lack of cold-tolerance, and agricultural policies resulted in lupin's demise so that by 1960s it has almost disappeared. Recently, there has been a renewed interest in using lupin as legume cover crop to meet N needs of succeeding crops. Lupin seed and plant tissue are characterized by low alkaloids ("Sweet") or high alkaloid ("Bitter"). It is desirable to have lupin lines with sweet seeds and bitter plant tissue since bitter plant tissue can act as a natural pesticide for disease and insect pests upon incorporation into the soil. We conducted biological N Fixation (BNF) studies with lupin lines varying in their alkaloid contents. The results indicated that high-alkaloid (Bitter) lupin lines had greater nodulation, a measure of biological N fixation, as compared with low-alkaloid (Sweet) lines. However, enough variation existed among the 97 germplasm lines to indicate that it may be possible to develop lupin lines with sweet seed and bitter plant tissue.

POST-HARVEST EXTENSION OF MARKET SEASON FOR POND-RAISED LIVE FRESHWATER SHRIMP IN GREENHOUSE TANKS. Brian L. Nerrie, Virginia Cooperative Extension, PO Box 9081, Virginia State University, Petersburg VA 23806. Freshwater shrimp (Macrobrachium rosenbergii) are fast-growing tropical organisms that are increasingly farmed in Virginia, especially in the tobacco growing counties. They are stocked as juveniles (~0.2 g) in late May-early June and harvested in late September-early October (>35 g). More than 80% of the freshwater shrimp harvested in Virginia are sold fresh on-ice to buyers on harvest day (\$16.00-25.00/kg) with some frozen for future sales. M. rosenbergii cannot survive water temperatures below 14°C. A demand exists for off-season live fresh shrimp. Shrimp harvested from ponds at VSU's Randolph Farm during the first week of October 2007 were either frozen (-10 °C) or transferred to aerated 1000 liter circular tanks. Tanks were equipped with substrate (orange-plastic fencing) and stocked at a low density of 25 shrimp per tank. Water temperature was maintained between 15-20 °C by aquaria heaters to reduce the need for feeding, and therefore minimize growth, molting and cannibalism. Shrimp were harvested after 150 days on 20 March and a taste test conducted to compare with previously frozen whole shrimp. A taste panel reported high acceptance and no differences (P>0.05) in taste, texture, and appearance between the previously frozen shrimp and fresh inventoried shrimp. Shrimp mortalities (>50%) were observed associated with high tank water temperature resulting from outside temperatures exceeding 30°C.

SURVIVAL AND GROWTH COMPARISONS OF CATFISH (ICTALURUS PUNCTATUS) FINGERLINGS IN CAGES OVER WINTER, SECOND-YEAR TRIAL WITH INDUSTRY APPLICATION. Scott H. Newton & Edward N. Sismour, Agriculture Research Station, PO Box 9061, Virginia State University, Petersburg VA 23806. Channel catfish (Ictalurus punctatus), an important fishery resource in Virginia, are regularly imported from southern states because of high demand. Previous results suggested that purchasing fingerlings in the fall and holding them over winter might be an effective management strategy to increase productivity and reduce mortality associated with transport and stocking in the spring. We conducted a second-year trial from November 2008 to April 2009 at VSU and at Gold Hill farm (GHF) in Buckingham County. Two groups of catfish purchased in 2008, one in April (spring) and the other in mid-October (fall), were compared. Fingerlings were restocked in mid-November at both locations into separate sets of three cages with 250 fish per cage. Total lengths and weights were measured on subsamples of 60 fish per cage at both stocking and harvest. Fish were fed a standard, floating-pellet ration when winter pond water temperature exceeded 10 °C. Catfish fingerlings in 2007-08 increased in weight by 17% for the spring group and 25% for the fall group, and both groups had high (>99%) survival. No increase in length or weight was observed in 2008-09 and survival was much lower, about 60% at both VSU and GHF for the spring catfish and about 30% and 70%, respectively, at VSU and GHF for the fall catfish. Fingerlings at VSU were affected by an out break of White Spot disease (Ich). Persistent, low (<10 °C) water temperature over the 2008-09 winter limited feeding opportunities resulting in poor growth and survival performance.

SURVIVAL AND GROWTH OF CHANNEL CATFISH (Ictalurus punctatus) FINGERLINGS IN CAGES WITH LONG-TERM ORAL ADMINISTRATION OF B-GLUCAN, AN INITIAL ASSESSMENT. Edward N. Sismour & Scott H. Newton, Agriculture Research Station, PO Box 9061, Virginia State University, Petersburg VA 23806. Bacterial disease is a major cause of financial loss to aquaculture producers. Antibiotics are typically used to control disease; however, only a small number are approved for food fish and legal restrictions limit usage. Immune system enhancement is an alternative approach with demonstrated benefits in numerous agricultural applications. β-1,3/1,6-Glucans are an integral part of the cell wall in bacteria, fungi, and some plants. The structural pattern is highly conserved and binding of these molecules to pattern recognition receptors in macrophage cell membranes upregulates nonspecific immune responses of these cells. Unlike antibiotics, β-1,3/1,6-glucan preparations are commercially available without restrictions on their application. The purpose of the present study was to evaluate the effect of β-1,3/1,6-glucan administered orally in the feed on the growth and survival of channel catfish fingerlings grown in cages. The glucan preparation used for this study was Agrastim®, and the basal ration was standard, commercially available, floating-pellet aquaculture feed. Two trials were conducted. In the first, 50 mg/kg and 100 mg/kg glucan dosages were compared to the basal ration and to the basal ration plus 1.2% agar top-coat used to facilitate application of glucan to the feed pellets. Treatments were isolated in separate ponds to prevent cross-contamination. The second trial compared the basal ration and 100 mg/kg glucan treatments in the same pond. At the dosages evaluated for this study, catfish survival

and growth were not improved over control treatments; however, beneficial effects of glucans have been reported at higher dosages and additional research is suggested.

DISEASES OF CAGE-REARED CATFISH. David Crosby1, Edward N. Sismour2 & Scott H. Newton<sup>2</sup>, <sup>1</sup>Virginia Cooperative Extension, Virginia State University, PO Box 9081, Petersburg VA 23806 and <sup>2</sup> Agriculture Research Station, PO Box 9061, Virginia State University, Petersburg VA 23806. Many producers of catfish in Virginia use farm ponds that are only suited for cage production. Catfish fingerlings are purchased from out-of-state producers and can spend over 20 hours in transit on a hauling truck. Transport time, crowding and associated factors may cause stress that potentially induces disease outbreaks. A study, initiated in 2007, is underway to assess and quantify factors contributing to catfish mortality following transport and cage stocking in the spring and the fall. Fish health assessments are conducted at the initial stocking and at 1, 2, and 3 weeks post stocking for which 60 fish are examined for diseases and external parasites on gills and skin. Not surprisingly, Henneguya sp was found at all initial stockings and during post stocking. Proliferative gill disease (PGD) was found at all initial spring stockings. However, fall stocking showed no clinical signs of PGD. Stocked catfish were infested with Ich within two weeks, except during fall 2008 when the catfish came down with Red Sore Disease. Trichodina sp and gill worms (Ligictaluridus sp.) were found in all spring initial stockings. The two fall stockings were free of most gill and skin parasites except for Henneguya. The 2007 spring stocking incurred 50% mortality attributed to Enteric Septicemia and columnaris. The 2007 fall stocking incurred only 1% mortality while the 2008 fall stocking had over 80% mortality. Fish stocked in the spring had more potential problems such as Trichodina, PGD and gill worms while fall fish had relatively few or any potential problems.

INSECTICIDAL ACTIVITIES OF PARTHENUM ARGENTATUM GRAY CRUDE METHANOLIC EXTRACT AND EXTRACT FRACTIONS ON ADULT GREENHOUSE WHITEFLY. F.D. Favi<sup>1</sup>, M. Tellez<sup>2</sup>, S.O. Duke<sup>2</sup> & M. Kraemer<sup>1</sup>, <sup>1</sup>Virginia State University, Agricultural Research Service, PO Box 9061, Petersburg VA and & <sup>2</sup>University of Mississippi, USDA/ARS National Center of the Development of Natural Products, PO Box 8084, Stoneville MS. P. argentatum (guayule) is a perennial plant introduced into the US for production of latex for medical use as an alternative to latex from the hevea tree. Guayule latex is used for medical purposes because it does not induce reactions as does hevea's latex. Guayule resin content represents 10% of whole plant-extract and has pest control activities. Methanolic extract and its fractions were used to assess resin toxicity on adult greenhouse whitefly. Ethyl acetate fractions were coded A-C, methanolic fractions coded D-H and N-P while methylene chloride fractions were named I-M. Extracts were either used to coat vials or applied to tomato leaf disk to study contact or oral toxicities respectively. ANOVA (SAS statistic package, 2004) was used to analyze the results. Contact toxicity of fractions (D-H and N-P) were significant with f = 4.78 df = 9, P < 0.001. However, fractions coded H and P had killed adult whiteflies by contact within 3 hours and had been selected as the most toxic fractions of guayule methanol extract. Fractions J and L have significantly good oral toxicity at a very low dose (f = 71.32, df = 4, p < 90.0001).

ANAGEMENT OF A NATIVE BEE FOR POLLINATION OF VIRGINIA APPLE ORCHARDS. Mark E. Kraemer, Chelsea Johnson & Françoise Favi, Agricultural Research Station, Virginia State University, Petersburg VA 23806. The blue orchard bee (Osmia lignaria Say) is native to most of temperate North America and known to be an excellent pollinator of apple and other rosaceous tree fruits. However, management techniques need to be developed before this bee can be used in orchards. Initial research identified natural enemies and the life cycle phenology of this bee in Virginia. In the last two springs these bees were tested in 3 apple orchards in Virginia and North Carolina using artificial nesting sites. Adult bees established nests in sheltered areas near the orchards and were able to increase their numbers by up to 3X in one season. Parasitism was not a significant problem although pollen mites were present and could be a secondary problem if larval mortality is significant and large amounts of pollen are left for pollen mites. Pesticide sprays did not appear to affect nest building activity but larval mortality was high (20%) in one orchard and may have been correlated with early season fungicide applications. Large amounts of apple pollen, up to 98%, were found in some nest cells constructed during apple bloom.

ANALYSIS OF ESTS FOR DIFFERENTIAL GENE EXPRESSION TO ANTHRACNOSE IN YAM (Diascorea alata L). Satya S. Narina<sup>1</sup>, Brian L. Sayre<sup>1</sup>, Shaukat M Siddiqi<sup>1</sup>, Alieu Sartie<sup>2</sup> & Robert Asiedu<sup>2</sup>, <sup>1</sup>Department of Biology, PO Box 9064, Virginia State University, Petersburg VA 23806 and <sup>2</sup>International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320 Ibadan, Nigeria. Molecular markers are ideal to investigate genetic effects on the resistance/susceptibility to disease. Simple Sequence Repeats (SSRs), repetitions of nucleotide motifs of 1-5 bases, are currently the markers of choice due to their abundant distribution in the genomes, and suitability for high-throughput analysis. Yam, (Dioscorea alata L), the main food source for over 100 million people in humid and sub-humid tropics, is vulnerable to anthracnose (Colletotrichum gloeosporioides) disease. This is one of the major limiting factors in the production of yam worldwide. A collaborative project between the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, Africa and Virginia State University, Petersburg, Virginia, USA was developed for genetic improvement and germplasm characterization of yams using molecular tools. Very limited sequence information is available from public genome databases. Total RNA was isolated from young leaves of resistant and susceptible genotypes and cDNA libraries corresponding to these two lines were constructed using Clontech's Creator SMART cDNA library construction kit. The libraries from the resistant and susceptible genotypes now have a total of 85,000 and 80,000 cDNA clones, respectively. These cDNA clones are currently being sequenced and nearly 80,000 EST sequences generated from this project are presented.

### Astronomy, Mathematics and Physics

QUANTITATIVE ANALYSIS OF BACKGROUND RADIATION PARTICLE TRACKS IN A LARGE DIFFUSION CLOUD CHAMBER USING "IMAGEJ" Robert Brik1 & David B. Hagan2, DIGITAL IMAGING TECHNIQUES. <sup>1</sup>Massachusetts Institute of Technology 02139 and <sup>2</sup>Science Museum of VA, 2500 W. Broad St., Richmond VA 23220. Cloud chambers are used to view radiation trails from solar, cosmic, and terrestrial sources. Image processing software was utilized to identify and quantify the differences between various particles. Alpha, Beta, Proton, and Muon particle images were analyzed to determine every particle's general geometry. Images were collected from the manufacturer, from photography, and from video. Each image was thresholded and then a statistical analysis was run. The particles' were found to have certain characteristics that made them distinct and easily identifiable by imaging software. Some of the more distinguishing traits were shown to be the ratio of area to perimeter, Feret's diameter, intensity skew, kurtosis, and circularity. Additionally, it was shown that a particle may produce various streaks, such as an almost circular point and a long straight line. These variations initially look like different particles; therefore, these similarities and variations have been catalogued as different types of the same particle. Using image analysis to isolate and count the particles allows for more efficient experimentation and a decrease in experimentation

SOLVING MATH WORD PROBLEMS WITH ENGLISH GRAMMAR. Richard A. Garrett & Richard S. Groover, Dept. of Math & Science, J. Sargeant Reynolds C.C., Richmond, VA 23228. A short term study in overall student effectiveness solving mathematical word problems took place during Spring 2009 academic semester. Developmental math students, who are prone to higher levels of math anxiety, were essentially learning new problem solving methods despite the fact that their course work was largely review. Students included were studying Algebra and were given a method for solving word problems in their classroom. The method involves breaking sentences down and analyzing their component pieces which are marked by grammar symbols. By properly analyzing these key points in every English sentence within the original problem, students were able to use their own knowledge of the language in order to create a reference map in order to translate English into Mathematics. A complete math problem is then constructed and solved by combining each piece. Encouragement in practicing this method resulted in overwhelmingly positive success rates regarding final answers and pattern recognition. Additionally, students who practiced this method showed higher confidence levels in Mathematics and became more self-motivated in solving problems.

INFRARED EMISSION PROPERTIES OF Nd: KPb<sub>2</sub>Br<sub>5</sub> FOR SOLID STATE LASERS. <u>C. Hanley</u><sup>1</sup>, E. Brown<sup>1</sup>, U. Hömmerich<sup>1</sup> & S. Trivedi<sup>2</sup>, <sup>1</sup> Department of Physics, Hampton University, Hampton VA 23668 and <sup>2</sup>Brimrose Corporation of America, Baltimore MD 21236. We report on the crystal growth and optical properties of Nd:KPb<sub>2</sub>Br<sub>5</sub> crystals for potential applications in mid-infrared (MIR) solid-state lasers. Following optical pumping at 800 nm, Nd:KPb<sub>2</sub>Br<sub>5</sub> exhibited a broad MIR

emission centered at  $\sim 5.25~\mu m$  with a bandwidth of  $\sim 730~nm$  at full width half maximum. For a moderate Nd<sup>3+</sup> concentration of  $\sim 5.5~x~10^{19}~cm^{-3}$ , the mid-IR was

predominantly due to transition  ${}^4I_{11/2} \rightarrow {}^4I_{9/2}$ . The peak emission cross-section obtained using the Fuchtbauer-Ladenburg method was  $\sim 0.6 \times 10^{-20}$  cm<sup>2</sup>. The MIR emission lifetime was measured to be  $\sim 50$  ms at room temperature. The radiative lifetime obtained from a Judd-Ofelt analysis was  $\sim 47$  ms, which indicates a MIR emission quantum efficiency near unity. The obtained spectroscopic results suggest the possibility of a MIR laser operating at  $\sim 5.5~\mu m$  based on Nd: KPb<sub>2</sub>Br<sub>5</sub>. However, further improvements in the purification and crystal growth of Nd: KPb<sub>2</sub>Br<sub>5</sub> are necessary to obtain laser quality samples. This study was funded in part by the National Science Foundation and Army Research Office.

MASSIVE AND MASSLESS BOSONS WITHOUT A HIGGS POTENTIAL. Joseph D. Rudmin, Integrated Science and Technology Dept., James Madison University, 800 S. Main St., Harrisonburg, VA 22807. In 1967, Steven Weinberg, Sheldon L. Glashow, and Abdus Salam published their "WGS Theory" which describes many observed fermions and bosons, and the symmetries of their interactions, and explains why the weak force which transforms those symmetries has short range while the electromagnetic force has long range. This theory offers a unified description of the weak force and electromagnetism. It received the 1979 Nobel Prize in Physics, partly due to its remarkable success in explaining the masses of many particles, and how they interact. However, the Higgs potential mechanism of WGS Theory predicts the Higgs boson, which has not yet been observed. This paper presents an alternative mechanism based on the effects of fermion polarization, for achieving the same result, and some further paradoxes and problems with both mechanisms.

MICROPROCESSOR ARITHMETIC--EFFICIENT LONG DIVISION AND MULTIPLICATION IN ANY NUMBER BASE WITHOUT A MULTIPLICATION TABLE. Joseph W. Rudmin, Dept. of Physics and Astron., James Madison Univ. 22807. Digital accuracy of standard numeric formats is limited by computer processors and by software. Such limitations in 2009 are presented. Methods of avoiding these limits and alternative arithmetic algorithms are presented, including the Russian Peasant Method, and two methods of long division, one of which is suitable for almost arbitrarily large numbers, and the other a new algorithm for long division which does not use trial divisors, and uses only the mathematical operations of shift, add, and subtract.

YORK RIVER, VA WATER TEMPERATURES AS SURROGATES FOR HISTORICAL WATER TEMPERATURES ELSEWHERE IN CHESAPEAKE BAY, VIRGINIA. Thomas C. Mosca III<sup>1</sup> & W.C. Coles<sup>2</sup>, <sup>1</sup>Dept of Mathematics, Rappahannock Community College and <sup>2</sup>Division of Fish and Wildlife – Department of Planning and Natural Resources. Temperature is one of the fundamental physical parameters of a body of water, and the rate and magnitude of marine chemical, physical and biological events are highly dependant upon water temperature. However, there are few long-term water temperature data sets in the Chesapeake Bay to establish

temporal trends. The water data maintained by the Virginia Institute of Marine Science (VIMS) at Gloucester Point, VA on the York River are the only continuous long-term water temperature data available for the Virginia portion of the Chesapeake Bay. The purpose of this paper is to present regression equations to predict water temperatures in eight regions of the Chesapeake Bay (upper and lower Virginia portions of the Bay, upper and lower portions of the James, Rappahannock and York rivers), from the VIMS temperature data. These regressions may be used to correlate temperatures with other documented data, and to fill holes in other data sets. We compared the monthly mean temperatures at VIMS to temperatures gathered on a monthly schedule in eight strata of Chesapeake Bay and the three major tributaries. The relationship between the VIMS pier temperatures and temperatures measured in other parts of Chesapeake Bay is very strong ( $R^2 \ge 95\%$ ), and therefore is a useful surrogate for temperature in ecological studies of other parts of the lower Chesapeake Bay.

CONCENTRATION DEPENDENT STUDIES OF THE LASER-INDUCED INFRARED EMISSION FROM KCI-NaCI TABLETS. O. Oyebola<sup>1</sup>, U. Hömmerich<sup>1</sup>, E. Brown<sup>1</sup>, Clayton S. C. Yang<sup>2</sup>, S. B. Trivedi<sup>3</sup>, A.C. Samuels<sup>4</sup> & A.P. Snyder<sup>4</sup>, <sup>1</sup>Department of Physics, Hampton University, VA 23668, <sup>2</sup>Battelle Eastern Science and Technology Center, Aberdeen, MD 21001, <sup>3</sup>Brimrose Corporation of America, Baltimore, MD 21236, and <sup>4</sup>Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010. Laser Induced Breakdown Spectroscopy (LIBS) has emerged as a widely used analytical technique to determine the elemental composition of chemical substances. Most previous LIBS studies were performed in the ultraviolet to nearinfrared (~200-980 nm) spectral region. In this work, results are reported on the extension of conventional LIBS to the mid-infrared (mid-IR) spectral region from 2-5 μm. Pumping with a pulsed Nd: YAG laser (1064 nm), mid-IR LIBS signatures were observed from solid KCl tablets at 2.72 μm, 3.15 μm, 3.77 μm, and 4.05 μm. In agreement with the NIST spectral database, the observed mid-IR emission lines were assigned to atomic transitions between higher lying energy states of neutral potassium (K) atoms. Further IR LIBS studies on KCl focused on the 2.72 µm emission line due to its relatively high intensity. A series of KCl-NaCl tablets with different amounts of potassium were prepared to determine the mid-IR LIBS detection limit of potassium. The preliminary results indicated a LIBS detection limit of 0.5wt% of potassium in the prepared KCl-NaCl samples. This study was funded in part by the Army Research Office.

STOCHASTICITY AND SPONTANEOUS SYMMETRY BREAKING IN THERMALLY INDUCED BENDING VIBRATIONS OF STRUCTURES. Anthony A. Teate, Department of Integrated Science and Technology, James Madison University, 800 S. Main St., Harrisonburg, VA 22807. A model is developed for thermally induced bending vibrations of uniformly heated beams in still air that assumes a non-linear dependence of the ratio of the unsteady to the steady-state component of the heat transfer coefficient on the velocity of the bending vibrations. This model also includes a stochastic driving term to account for the effects of thermal fluctuations in the ambient air and the concomitant random impacts on the beam and yields a non-linear, stochastic description of the heated vibrating beam for the

dimensionless displacement S(t) of the form:

$$\frac{d S(t)}{dt} = \alpha_1 S - \alpha_2 S^3 + \widetilde{F}(t) \quad \text{where} \quad \widetilde{F}(t) \quad \text{is purely random,}$$

stationary, Gaussian, process with zero mean, and represents the effects of the amplitude fluctuations due to the random impacts of the ambient air on the beam and

where the  $\alpha_i$  are constants dependent upon the thermal and modal bending moments, damping ratio and the phase difference between the velocity fluctuation and thermal bending moments. We solve the stochastic differential equation by constructing a symmetry breaking, bi-stable *Thermal Potential Energy Function*, a Lyapunov global stability function which permits a general investigation and analysis of the system's stability and the effects of pressure on thermally induced bending vibrations.

ASTRONOMICAL POLARIMETRY AT VIRGINIA MILITARY INSTITUTE. Gregory A. Topasna, Daniela M. Topasna & Gerald B. Popko, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. We present current work on the design, construction, and testing of a two-beam optical polarimeter to be used with the 20-inch telescope at the Virginia Military Institute observatory. The basic operation of the device will be discussed as well as results which demonstrate the two-beam method in the laboratory. Issues regarding automation and data handling as well as planned observations will be presented.

# **Biology**

DELAYED TREATMENT WITH SILDENAFIL ATTENUATES ISCHEMIC CARDIOMYOPATHY. V.O. Chau, F.N. Salloum & R.C. Kukreja. Cardiology, Virginia Commonwealth Univ., Richmond, VA 23298. We previously showed that chronic inhibition of phosphodiesterase-5 (PDE-5) with sildenafil immediately after permanent occlusion of the left anterior descending coronary artery (LAD) limits myocardial infarction (MI)-induced heart failure (HF) in mice. To mimic more clinical scenarios, we hypothesized that chronic treatment with sildenafil beginning at 3 days post MI would also preserve LV function and reduce HF progression. Adult male ICR mice underwent MI by permanent ligation of the LAD after baseline echocardiography was performed. Three days post MI, a repeat echocardiography was conducted. Mice with LV fractional shortening (FS) less than 25% received sildenafil (21 mg/kg; ip; BID, Group I) or volume-matched saline (Group II) for 25 days. At the completion of 28 days following MI, the mice underwent a repeat echocardiography prior to sacrifice. Group I expressed less LV dilatation than group II, and group I showed better contractility as compared with group II. LV enddiastolic diameter (LVEDD), increased from a baseline value of  $3.4 \pm 0.1$  mm to 4.2 $\pm$  0.1 at 72 hr post MI. At 28 days post MI, LVEDD was increased to 5.2 $\pm$ 0.1 mm for group II, as compared 4.6±0.1 mm in group I (P<0.05 vs. Group II). Fractional shortening decreased from a baseline value of 47±1% to 19±1% at 72 hr following MI. At 28 days post MI, FS was 21±1% for group I and 13±1% for group II, (P<0.05 vs. Group II). For the first time, these results show that chronic sildenafil treatment initiated at 3 days post MI attenuates ischemic cardiomyopathy by limiting LV dilatation and preserving FS. Sildenafil may be a promising therapeutic tool for prevention of HF in patients with MI.

CONTROLLED ASSEMBLY OF NANOSCALE PROTEIN/DNA "BASKETS" FOR THE IN VIVO DELIVERY OF siRNA PARTICLES. A.C. Zirzow, C.B. Smith, and A.V. Baranova, Dept. of Mol. and Microbiol., R. Couch and A.S. Patanarut, Dept. of Chemistry, George Mason Univ., Fairfax VA 22030. The purpose of this research is to develop a novel vector for the in vivo delivery of siRNA particles. In attempt to overcome current siRNA transfection problems of cytotoxicity, physical size of vector, instability of siRNA, and rapid clearance from the bloodstream, we developed the concept of a nanoparticle comprised of an outer cage made of protein and DNA (DNA "basket") that is capable of carrying siRNA cargo. This investigation demonstrates that protein/DNA interactions can be exploited to form DNA "baskets" with a stable mean size distribution. Derinat (Деринат), a DNA-based immunomodulator, is employed as the DNA component of these DNA "baskets". When prepared in the absence of protein, Derinat retains a stable mean size distribution of  $762.4 \pm 7.26$  nm. When this DNA is vacuum concentrated with a specific quantity of bovine serum albumin (BSA), the mean size distribution can be significantly reduced by up to 98%. Reduced mean diameters of the DNA/BSA complex may allow for more efficient in vivo cellular uptake. The next step in this investigation is to determine if a DNA/BSA complex can contain and hinder the degradation of GFP specific siRNA particles for in vivo delivery into GFP mice. The degradation of fluorescently tagged/quenched siRNAs will be monitored in vitro in nuclease-containing serum and in vivo. This novel approach to siRNA based therapy may minimize side effects, increase cellular uptake, and provide a scaffolding upon which ligands may be attached to direct siRNA to tissues of interest.

A SEARCH FOR KCNRG MUTATIONS IN MULTIPLE MYELOMA CELL LINES. Stephanie L. Coon & Aybike Birerdinc & Ancha Baranova. Dept. of Mol. and Microbiol., George Mason Univ., Fairfax VA. 22030. Deletions and or rearrangements on chromosome 13q14.3 are observed in more than half of multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) cases and are also frequently seen in other hematopoietic malignancies. The minimal common deleted region (CDR) in MM cells contains candidate tumor suppressor gene KCNRG (potassium channel regulating gene), the transcript of which suppresses Kv channels associated with the proliferation of lymphocytes. KCNRG exerts growth suppressive and pro-apoptotic effects in HL-60, LnCaP and RPMI-8226 cells. In this study we sequenced KCNRG gene in three multiple myeloma cell lines. We found that RPMI-8226 cell line contains a delT mutation in the core promoter initiator element. Deletion of T decreases matrix similarity of the match from 0.945 to 0.941, and, therefore, might negatively influence expression of KCNRG in RPMI-8226 cells. This suggests that KCNRG expression may be negatively influenced in this model line. The haploinsufficiency of KCNRG might be relevant to the progression of CLL and MM at least in a subset of patients. This research was performed under NIH R1R15CA113331-01, RFFI 07-04-00379-a, 07-04-12232-ofi, and 04-04-08154-ofi.

KPP: KEGG PATHWAY PAINTER. Ganiraju Manyam, Vikas Chandhoke & Ancha Baranova, Department of Molecular and Microbiology George Mason University, Fairfax, VA 22030. High-throughput technologies became common tools to decipher changes of gene expression (GE) patterns. Functional analysis of GE patterns is a daunting task as it often requires recourse to the public repositories of biological knowledge. On the other hand, in many cases researcher's inquiry can be served by a comprehensive glimpse. The KEGG PATHWAY database is a compilation of manually verified maps of biological interactions presented as a set of pathways related to signal transduction and other cellular processes. Rapid mapping of the differentially expressed genes to the KEGG pathways may assist in evaluation of the functional relevance of the results from microarrays and other high-throughput technologies. Web based graphic tool KEGG Pathway Painter (KPP) provides fast and comprehensive visualization of the changes in GE patterns by color-coding pathways from the KEGG database using user defined sets of the candidate genes accompanied "overexpressed" or "underexpressed" marks, for example, those generated by freely available microarrays. KPP is and can be accessed http://www.cos.gmu.edu/~gmanyam/kegg/. The study was supported by NIH 1R15CA113331-01 and Service GRA of College of Science, George Mason University.

CUG2 (C6ORF173) IS A NOVEL ONCOGENE INVOLVED IN BREAST CARCINOMA. Elizabeth D. Nohelty<sup>1</sup>, M. Skoblov<sup>2</sup>, V. Kuznetsov<sup>3</sup>, & A. Baranova<sup>1,2</sup>, <sup>1</sup>Department of Molecular and Microbiology, George Mason University, Fairfax, VA; <sup>2</sup>Russian Center for Medical Genetics, Moscow, Russia, <sup>3</sup>Bioinformatics Institute, Singapore. The genetic mechanism of the aggression of breast carcinoma has been a topic of research efforts since its discovery in the human population. Previous studies showed that an increase of C6ORF173 expression is associated with shorter survival after breast carcinoma diagnosis (Ivshina et al., 2007). Using Real-Time PCR ready TissueScan Cancer qPCR Arrays comprised of normalized cDNA prepared from pathologist-verified breast carcinoma samples we demonstrated that expression levels of C6ORF173 are significantly (P < 0.013) higher in Grade 3 breast carcinoma tumors as compared to Grade 2. C6ORF173 has been cloned into the pCDNA3.1 expression vector and stably transfected into HCC2157, NM2C5 and MCF-10A breast carcinoma cell lines. Analysis of transfected NM2C5 cells demonstrated a statistically significant increase of proliferation after 48 hrs of incubation with BrDu (P < 0.00016) as well as an increase in migration and invasion, while apoptosis ability of NM2C5 was not changed. Weak homology of C6ORF173 to a known downregulator of transcription (DR1) suggests its involvement in gene expression regulation in a broad sense. If the C6ORF173 gene indeed plays a large role in the aggression of breast carcinoma, it is possible that a genetic screen can be implemented to further improve the predictive diagnostic and treatment of breast carcinoma.

FRANCISELLA NOVICIDA FORMS IN VITRO BIOFILMS MEDIATED BY AN ORPHAN RESPONSE REGULATOR. Meghan W. Durham-Colleran, Anne Brooks Verhoeven, & Monique L. van Hoek, Department of Molecular and Microbiology, National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, VA 20110. Francisella tularensis is associated with water and waterways,

and infects many species of animals, insects, and protists. The mechanism Francisella utilizes to persist in the environment and in tick vectors is currently unknown. We have demonstrated for the first time that Francisella novicida, a model organism of F. tularensis, forms a biofilm in vitro. Selected F. novicida transposon mutants were tested for their ability to form biofilm compared to the wildtype F. novicida strain. Mutation of the putative qseB gene led to an impairment in the ability to form biofilm with no impairment in bacterial growth. A qseC mutant had impaired growth, but demonstrated a marked impairment in biofilm production. Mutation in cap C affected both bacterial growth and biofilm formation, but no biofilm production impairment was seen with capB or pilE mutants. A deletion mutant in the orphan response regulator FTN 1465, which we propose is the putative QseB, formed significantly less biofilm than the wildtype. When FTN 1465 was complemented back into the deletion mutant, biofilm formation was restored. Thus, the orphan response regulator FTN\_1465 is an important factor in biofilm production in vitro in F. novicida. These results demonstrate that Francisella species are able to form biofilms in vitro, suggesting that biofilm formation may be important for the life cycle of this organism in the environment or possibly in the tick vector.

TWO MINOR SPECIES AS DOMINANTS IN AN OLDFIELD RODENT COMMUNITY. Robert K. Rose, Dept of Biol. Sci., Old Dominion Univ., Norfolk, Virginia 23529-0266. Oldfields are early stages in secondary succession dominated by herbivores, including three species of common rodents (meadow voles, cotton rats, marsh rice rats). In two oldfield community studies in eastern Virginia, these species comprise >90% of captures, but all were absent in another oldfield habitat, created after logging of a pine forest, clearing, and mechanical planting of pines. There the grasses, sedges, and spikerushes provided habitat to support populations of two minor herbivorous rodents, southern bog lemming (Synaptomys cooperi) and woodland vole (Microtus pinetorum), which reached densities of 15/ha and 35/ha, respectively, across an 18-month capture-mark-release study. Besides the >450 captures of these two dominants, 7 captures of harvest mice were recorded, but no meadow voles, cotton rats, or marsh rice rats. Some studies conducted elsewhere suggest that southern bog lemmings lose in competition with meadow voles, but the reasons for the presence or absence of a species cannot be determined without experimentation.

BODY SIZE AND GROWTH PATTERNS OF MICROTUS PENNSYLVANICUS (ORD.) IN CHESAPEAKE, VIRGINIA. Sara E. Bell & Robert K. Rose, Dept. of Biol., Old Dominion Univ., Norfolk VA 23529-0266. From Dec 2002-Feb 2008, we did a capture-mark-release study on 2 Chesapeake, VA populations of meadow voles (Microtus pennsylvanicus). The study sites were effectively 1 ha grids in oldfields. We put 2 modified live Fitch-type traps at 12.5 m intervals and trapped on the grids in monthly 3-day sessions. In northern North America, voles experience autumn and winter weight loss, demonstrate delayed growth and sexual maturation in autumn-born young, have lifespans under 15 weeks, and typically weigh no more than 55 g. Chesapeake voles experienced no seasonal weight loss, exhibited no delayed growth or sexual maturation, lived over 20 weeks, and nearly 20% weighed over 70 g. The longest-lived vole was an 80-week-old male. The heaviest individual voles were over

90 g and present in late autumn and winter at both sites. Breeding occurred year-round. Thus, meadow voles in eastern Virginia contrast sharply with more northerly populations in many aspects of their biology.

NATURAL GENETIC VARIATION IN METABOLIC RATE AND ACTIVITY IN WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) IN RELATION TO GENETIC VARIATION IN REPRODUCTIVE PHOTORESPONSIVENESS. Madelyn G. Crowell<sup>1</sup>, Paul Kaseloo<sup>1</sup>, and Paul Heideman<sup>2</sup>, <sup>1</sup>Dept. of Biol., Va. State Univ. Petersburg VA 23806 and <sup>2</sup>The Coll. of William and Mary, Williamsburg VA 23185. A naturally-variable life history trait with underlying physiological variation is the photoperiodic response of many temperate zone rodents, including white-footed mice (Peromyscus leucopus). Male P. leucopus were obtained from a short photoperiod responsive (R) line, selected for reproductive suppression in short-day conditions (SD) and a non-responsive (NR) line selected for reproductive maturity in SD. NR mice consume ~50% more food than R mice, but have no significant difference in body mass. We quantified differences in the energy budgets of these lines through respirometric measurements at thermoneutral temperature. Basal metabolic rate (BMR) was significantly greater in NR than R mice. In addition, NR mice engaged in significantly more daily activity. No significant difference in mass of major metabolic organs or dry mass digestibility of food was found between lines. The increased BMR and sustained metabolic rate in NR mice was correlated with testis size, but not with major central organs. The genetic difference in intake requirements between lines was great enough to be reasonably attributable to selection on the natural genetic variation in BMR and activity in the wild source population. These findings are consistent with differences in thyroid-related hormone activity which recent findings suggest mediate the response to photoperiodic reproduction. This study was funded in part by Howard Hughes Medical Institute.

HABITAT AVAILABILITY AND SPECIES-AREA RELATIONSHIPS OF INDO-PACIFIC SHORE BIOTA. Jonnell C. Sanciangco & Kent E. Carpenter, Department of Biological Sciences, Old Dominion University, Norfolk VA 23529. In marine biogeography, the measure of available habitat is a key factor in identifying the distribution patterns of species richness. The species-area relationship (SAR) has been widely used to infer this correlation of species richness to available habitat. While several studies have shown that larger habitat areas account for a higher number of species, the factors influencing the species richness and the amount of variation have yet to be identified. In this study, the SAR of Indo-Pacific shore biota was tested using the habitat diversity index (H) and coastal length (CL) as functions of area. The H was calculated using the Shannon-Weiner formula with areas of coral reefs, seagrasses, mangroves, and soft bottom as the parameters. Species distribution maps of 6830 marine shore biota (fishes, molluscs, and crustaceans) were created using Geographic Information System (GIS). In addition, multiple GIS tools, extensions and scripts were used to create a 200 meter bathymetry shapefile which was divided into three scale sizes of equal area sections (100, 500, and 1000) to minimize area effect. Values of H, CL, and species richness (S) were identified in each section. Linear regression analyses were performed for S vs H, S vs CL, and S vs H + CL. Results showed significant

differences (<0.001) for all relationships in all scales. H accounts for more variation (14.3-19.3%) than CL (7.6-13.2%), suggesting H as a better predictor of the species richness. Results are portrayed spatially using GIS, where species distribution of marine biota can be easily identified in the map. These results are used to assess the conservation status of marine species and to identify priorities for management.

SEASONAL PHYTOPLANKTON POPULATIONS IN BACK BAY, VIRGINIA Nathan Bowman, Todd Egerton & Harold Marshall, Department of Biology, Old Dominion University Norfolk, Virginia 23529. Back Bay is a flat-bottomed, shallow water ecosystem separated from the Atlantic Ocean by a narrow zone of marshlands, dunes, and residential development. Water depth in the Bay is influenced by the prevailing northeast winds, which may alter the depth in near shore regions by as much as 1.0 m. Presently, the only salt water entry to Back Bay is wind forced, passing into the Bay through a narrow channel from a large sound to the south. Back Bay is classified as a temperate, oligohaline estuary containing salinity ranges from 1.0 - 1.9, and has gained regional interest and concern by state and federal agencies regarding changes to its ecological status. A specific objective of the Back Bay National Wildlife Refuge is to reduce the impact of various environmental factors such as nutrient loading and high turbidity levels that would deteriorate its natural setting. One of the most sensitive components within this habitat to environmental changes is the phytoplankton, which may be used as an ecological indicator of Back Bay's eutrophic status. During the course of one year, the freshwater reaches of the Back Bay oligonaline estuary were sampled bimonthly at a series of six stations comprising the entirety of the bay. The goal of this study is to determine if the specific water quality conditions in this habitat are associated with seasonal changes in the abundance and dominance of specific phytoplankton components, including a changing seasonal flora and phytoplankton categories that occurred between September 2006 and September 2007.

THE BIOLOGICAL ACTIONS OF HYDROXY-CIS-TERPENONES. Tristan A Hayes, Lin Zhang, Qibing Zhou, Ghislaine Mayer & Jennifer Stewart, Virginia Commonwealth University. Hydroxy-cis-terpenone (HCT) was synthesized by Dr. Qibing Zhou in the VCU Chemistry Department. HCT is converted to oxidized HCT (OHCT) in aqueous media. Previous studies demonstrated that micromolar concentrations of HCT and oxidized HCT (OHCT) protect human liver cells from aflatoxin. Additionally, Dr. Ghislaine Mayer in the VCU Biology Department found that nanomolar concentrations of OHCT kill all blood stages of Plasmodium falciparum, the parasite responsible for most cases of human malaria. The goal of this project was to investigate mechanisms of HCT actions. Because binding of aflatoxin to microsomal proteins is needed for activation of aflatoxin, we investigated effects of HCT on binding of <sup>3</sup>H-labeled aflatoxin to human liver HepG2 cell membranes and human liver microsome proteins. Effects of various concentrations of HCT on protein binding of <sup>3</sup>H-labeled aflatoxin were measured at various times from 30 sec to 10 min. The data indicated that HCT at 20 - 40 uM decreased binding of <sup>3</sup>H-labeled aflatoxin to proteins within 30 sec. Binding was not ATP-dependent. Low concentrations of HCT (< 10 uM) did not affect binding to cellular proteins. These findings suggest HCT may reduce aflatoxin toxicity by reducing aflatoxin binding to liver cell proteins. This

work was supported by the Jeffress Memorial Trust J-849, NSF Grant MCB-013149, and the Advisory Committee for Undergraduate Research and Creative Scholarship.

INVESTIGATION OF DUAL PHENOTYPE GABA/GLUTAMATE NEURONS IN ZEBRAFISH. Lauren P. Bell & Dianne M. Baker, Dept. of Bio. Sci., Univ. Mary Washington, Fredericksburg, VA 22401. The purpose of this research was to investigate the presence of neurons expressing both the inhibitor neurotransmitter, GABA, and the excitatory neurotransmitter, glutamate, in zebrafish. This novel class of neurons has been recently identified in rodents, and increasing evidence suggests they play a role in GnRH signaling. To determine the presence of dual phenotype neurons, we developed in situ hybridization (ISH) probes for mRNA encoding proteins involved in GABA synthesis (GAD67) and glutamate transport (VGLUT 2.1 and 2.2). To test theses probes, we performed single-label ISH on whole-mount larva and on adult brain sections. These tests provided consistent evidence that the GAD67 riboprobe is functional in both larval and adult brain tissue. However, the results of ISH using the VGLUT 2.1 and 2.2 riboprobes were inconsistent. Further optimization of ISH conditions is necessary before dual-label ISH can be used to test for presence of dual phenotype GABA/glutamate neurons in zebrafish.

CHARACTERIZATION OF THE EXPRESSION OF CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 1 IN ZEBRAFISH. Colby S. Croft & Dianne M. Baker, Dept. of Bio. Sci., Univ. Mary Washington, Fredericksburg, VA 22401. The objective of this research was to characterize the spatial and temporal expression of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) in zebrafish, Danio rerio. To assess temporal expression, we first cloned four fragments of the coding region of the zebrafish CEACAM1 gene into Escherichia coli so that the CEACAM1 sequence in our strain of zebrafish could be determined. Then, real time primers and probes were designed based on this sequence. Real time PCR was performed on cDNA synthesized from mRNA isolated from embryonic and larval zebrafish at 24, 48, and 72 hours post-fertilization (hpf), and one week post-fertilization. We found a progressive increase in CEACAM1 mRNA expression over a one week period, with levels significantly higher at one week postfertilization than at 24 hpf (p<0.05). To characterize the spatial expression of CEACAM1 in zebrafish, we synthesized sense and antisense DIG-labeled RNA probes for in situ hybridization (ISH). The results of the ISH did not reveal a tissue-specific pattern of expression, as both the sense and antisense probes bound nonspecifically in embryos and larvae.

THE CLONING AND CHARACTERIZATION OF A PUTATIVE TYPE VI SECRETED CONSERVED PROTEIN (PA0083) FROM *Pseudomonas aeruginosa*. Nasira M. Rushdan<sup>1</sup>, William B. McVaugh<sup>1</sup>, Thomas M. Kerkering<sup>2</sup>, & Jayasimha Rao<sup>1,2</sup>, <sup>1</sup>Biomedical Sciences Department, Jefferson College of Health Sciences, Roanoke, VA 24013, <sup>2</sup>Infectious Diseases, Virginia Tech Carilion School of Medicine, Roanoke, VA 24013. Differentially expressed proteins from *Pseudomonas aeruginosa* have been identified based on a two-dimensional (2-D) gel electrophoresis analysis. The pattern was compared between two genetically similar but phenotypically distinct

P. aeruginosa strains, non-mucoid 383 and mucoid 2192, which were isolated from the same CF patient. In this study, a protein spot was cored based on its elevated expression pattern in the non-mucoid 383 strain. The cored spot was subjected to tandem-mass spectrometry, and the identified peptide sequences were classified as PA0083, an unknown hypothetical protein from the PAO1 genome. Bioinformatics analysis predicted that PA0083 has IcmF-associated homologous protein-related loci with a type VI secretion system. PA0083 was cloned into the 6x his-tagged expression system using the Gateway cloning method and recombinant PA0083 protein was produced in Escherichia coli. Studies are underway to determine whether PA0083 protein does have a role in pathogenesis.

CHARACTERIZATION OF PUTATIVE CLONING AND **SECRETORY** HYPOTHETICAL PROTEIN (PA0460) FROM PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES FROM A CYSTIC FIBROSIS PATIENT. Joel R. Saul<sup>1</sup>, William B. McVaugh<sup>1</sup>, Thomas M. Kerkering<sup>2</sup>, & Jayasimha Rao<sup>1, 2</sup>, <sup>1</sup>Biomedical Sciences Program, Jefferson College of Health Sciences, Roanoke, VA 24013, and <sup>2</sup> Infectious Diseases, Virginia Tech Carilion School of Medicine, Roanoke, VA 24013. Pseudomonas aeruginosa is an important pathogen causing chronic lung infections in patients with cystic fibrosis (CF). Differentially expressed proteins in P. aeruginosa have been identified based on two-dimensional (2-D) gel electrophoresis analysis. The pattern was compared between two genetically similar but phenotypically distinct P. aeruginosa strains, non-mucoid 383 and mucoid 2192, which were isolated from the same CF patient. In this study, a protein spot was selected based on the expression pattern, which was seen only in the mucoid 2192 strain. The cored spot was subjected to tandem-mass spectrometry or mass mapping, and peptide sequences were classified as PA0460, an unknown hypothetical protein from the PAO1 genome. Bioinformatics analysis showed that PA0460 has a putative signal sequence with a cleavage site after 22 amino acids, suggesting that PA0460 could be a secretory protein. PA0460 was cloned into the 6x His-tag expression system using the Gateway cloning method, and recombinant PA0460 protein was produced in Escherichia coli.

EFFECTS OF HYPOXIA ON MOUSE CARDIAC MUSCLE MORPHOLOGY. Quincey Garcia<sup>1</sup>, Lei Xi<sup>2</sup> & Kathryn E. Loesser-Casey<sup>1</sup>, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA, 22401 and <sup>2</sup>Dept. of Cardiology, Virginia Commonwealth Univ., Richmond, VA 23298. Systemic hypoxia (SH) can be caused naturally by high altitudes or as a result of a disease process such as sleep apnea or heart failure. Regardless of the initial cause, SH can interfere with a person's oxygen supply resulting in the cells' inability to make ATP by oxidative phosphorylation. However, studies have shown that SH may also have beneficial effects, such as a lower incidence of heart attacks. The exact mechanism of this protection has not been clearly defined and few morphologic studies have been done to study the effect of SH on cardiomyocytes. The goal of this study was to begin characterizing the effect. Three ICR mice were subjected to 2 cycles of systemic hypoxia using a normobaric plexiglass chamber with 10% oxygen. After 4 hours of hypoxia, the animals were allowed to recover for 24 hours and the cycle repeated. The hearts were perfusion fixed, embedded in wax, sectioned and stained. At least 3 sections from each of 3 control

mice and the 3 SH mice were photographed and the areas and diameters of the cells were measured using Image J. The mean diameter of the cardiomyocytes decreased by 16% following SH compared to the control cells. Although there appeared to be a similar decrease in area, a Student's T-test determined that the means of the control and treated groups were not different (p=0.056). Other cells, such as neurons, have been shown to decrease in size after SH but whether the mechanism is similar in cardiomyoctes is unknown.

THE EFFECT OF GLIMEPERIDE AND GLIPIZIDE ON MYOCARDIAL PROTECTION IN STEM CELLS. Jessice R. Themak & Kathryn E. Loesser-Casey, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA, 22401 Sulfonylureas are hypoglycemic drugs often used to treat patients suffering from diabetes mellitus. They work by binding to and blocking ATP sensitive potassium channels in β-cells of the pancreas thus regulating the release of insulin. These K<sup>+</sup> ATP channels are also present on the membranes of cardiomyocytes and the opening of these channels can have a cardioprotective effect. However sulfonylureas may ameliorate the beneficial effects of K<sub>ATP</sub> channels openers and thus prevent myocardial preconditioning, increase infarct size, and reduce time before ischemic contracture develops. A newer sulfonylurea, glimepiride may be more effective in treating diabetes mellitus due to its lower binding affinity for KATP channels in cardiac cells which suggests that ischemic preconditioning can be maintained with pre-treatment of this drug. To further investigate the effects of glimepiride on myocardial preconditioning, brown adipose tissue-derived stem cells were pretreated with glimepiride and glipizide and then exposed to hypoxia for a period of 18 hours. The mean number of surviving cells appeared to be greater in those cells pre-treated with glipizide when compared to the control. However, statistical analysis revealed that glipizide or glimepiride had no effect on myocardial preconditioning. Further study should be conducted to look at the effects of sulfonylureas on myocardial metabolism as well as action potentials which also seem to play an important role in preconditioning.

## **Biomedical and General Engineering**

(No Abstracts Submitted)

### **Botany**

PHYLOGENY OF THE LEGUME GENUS ARACHIS USING NUCLEAR AND PLASTID SEQUENCE INFORMATION. S.A. Friend<sup>1</sup>, D. Quandt<sup>2</sup>, & K.W. Hilu<sup>1</sup>. Dept. of Biological Sciences, Virginia Tech., Blacksburg, VA 24061 and <sup>2</sup>Rheinische Friedrich-Wilhelms-Universität, Nees-Institut für Biodiversität der Pflanzen, Meckenheimer Allee 170, D-53115, Bonn, Germany. The peanut genus Arachis L. (Fabaceae) contains 80 species and is native to South America. Krapovicaks and Gregory (1994) divided Arachis into nine sections based on morphology, geographic distribution and cytogenetics: Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoides, and Triseminatae. The largest of these, section Arachis, has been further subdivided into three genomes (A, B, and D) based on cytogentics. While this genus contains the crop peanut, a

comprehensive phylogeny for Arachis is lacking. Sequence information from plastid trnT-trnF and nuclear ITS from a total of 47 species representing all nine sections have been used to reconstruct the first molecular phylogeny for the entire genus, with Chapmannia and Stylosanthes as outgroup. Our results from ITS sequences show that the allotetraploid species A. hypogaea and A. monticola contain alleles that represent the proposed A and B genome progenitors, A. duranensis (A) and A. ipanensis (B). The sections Caulorrhizae and Triseminata are monophyletic, thus validating these sections. Arachis macedoi (section Extranervosae) is the first branching lineage, while the remaining species of this section are resolved in a terminal clade. Majority of the Arachis species are resolved in one of three main clades. The terminal clades (Arachis I and II) are comprised of section Arachis species and other sections placed within these.

STUDIES OF THE HEPATICAE AND ANTHOCEROTAE ALONG HAZEL RUN IN ALUM SPRING PARK, FREDERICKSBURG, VA - COMPARISON OF SAMPLING METHODS. Stephen W. Fuller & Emily Noordhuizen, Dept. of Biological Sciences, Univ. of Mary Washington, Fredericksburg, VA 22401. Initial sampling, carried out in the fall of 2008, used a complete sampling strategy to determine what liverworts and hornworts live in this habitat. To insure that the survey was as exhaustive as possible, it was repeated. In the spring of 2009 a stratified random sampling method was used on the same stretch of the stream to determine the comparative amount of labor involved and how efficacious this method would be in finding these species. The fall sampling was done to identify species which could be observed on botany field trips, whereas the spring sampling objective was to decrease the time and effort involved in sampling the entire course of the Hazel Run creek. Twelve species of liverworts and hornworts were found in the complete sampling, which required approximately 27 hours of field work. The random sampling technique revealed 8 species; it required about 8 man-hours to measure the strata, and about 20 man-hours of additional field work to locate the random collection sites and examine them for potential specimens. The stratified random sampling in the second survey proved to be less complete and just as time consuming as the complete sampling protocol; therefore, stratified random sampling would not seem to be a method of choice if one were interested in maximizing the number of liverwort and hornwort species found in this type of habitat, or in decreasing the effort involved in a survey of these plants.

A FLORISTIC SURVEY OF THE SMITH CREEK RESTORATION AREA IN ROCKINGHAM COUNTY, VIRGINIA. Karl V. Gorzelnik & Conley K. McMullen, Dept. of Biology, James Madison Univ., Harrisonburg, VA 22807. A floristic survey is being conducted as part of an ongoing collaborative project to monitor various aspects of a stream restoration project. The study area lies along part of Smith Creek in Rockingham County, Virginia. The intent of this project is to survey and catalogue vascular plants as a former pasture is being reclaimed. The results from the present survey will ultimately be compared with those obtained during later stages of the restoration, with the expectation of seeing an increase in diversity over the years. Plants are being collected and identified, and voucher specimens are being mounted

and stored at the James Madison University Herbarium (JMUH). From March 2008 through April 2009, 94 species have been collected and identified to species (32 families, 73 genera). Of these 94 species, 46 are native and 48 are introduced.

THE FLORA OF VIRGINIA PROJECT: A 2009-2009 UPDATE. Marion B. Lobstein, Dept. of Biology, Northern Virginia C.C., Manassas, VA 22205. Virginia, for its landmass, has the most diversity of vascular plant species of any state in the United States. It had the first flora, the Flora Virginica in 1739, yet does not have a modern flora. The Virginia Academy of Science for over eighty years has supported efforts to produce a modern Flora of Virginia. In 2001 the Foundation of the Flora of Virginia, Inc, was formed in 2001 and in May 2002 received 501(c) 3 status. Progress continues to be made on the efforts to develop a Flora of Virginia including fund-raising and public outreach efforts. Work on the content of the Flora of Virginia including the nearly 300 of the core illustrations have been commissioned, completed, and funded by VAS funds. A second Gwathmey Trust grant and one from the Robins Foundation have been awarded to the project this past year. The Academy, including the Fellows, continues to provide essential support including financial for this Project. Other progress includes completion of treatments of the dichotomous keys of 170 of the 201 vascular plant families in Virginia and the first step in developing species and genus descriptions has been completed. The second step of herbarium work on descriptions is 74% complete and the third and final step of species genus descriptions is 52% completed. The projected publication date is late 2012 or early 2013.

PLEOMORPHIC CHLOROPHYTES: A CHALLENGING PHENOMENON IN SPECIES SYSTEMATICS. Harold G. Marshall, Dept. Biological Sciences, Old Dominion Univ., Norfolk, VA, 23529-0266. The identification of chlorophytes is commonly based on standard keys that portray a representative figure and its dimensions. It is assumed that features given are stable and are major criteria used in identification. However, these illustrations commonly come from preserved field samples showing one stage in the species life cycle. Any deviation from these features has often led to establishing a new species, or varieties and forms of an existing species. Yet many of these taxa are pleomorphic, having multiple forms with morphological features that differ from the standard illustration in identification keys. To know these stages living specimens have to be studied. Unfortunately, little is known regarding the life cycle of many of these taxa, and life stages from a variety of chlorophytes have been mis-identified. These life cycle stages are under genetic control, with interaction from a variety of environmental factors (e.g. water temperature, nutrient concentrations and ratios, light intensity, predation, and other water quality variables) influencing the onset and duration of this development, indicating phenotypic plasticity is common among the chlorophytes. The study of living specimens and their life cycle is necessary to recognize this variability, plus the use of molecular genetic protocols to verify speciation among these taxa.

DARWIN'S GALÁPAGOS PLANT COLLECTIONS. Conley K. McMullen, Dept. of Biology, James Madison Univ., Harrisonburg, VA 22807. From 15 September to 20 October 1835, while serving as naturalist aboard HMS *Beagle*, Charles Darwin was

presented with an opportunity to collect plants from four islands within the Galápagos archipelago (Chatham, Charles, Albemarle, and James). Although not the first scientist to take plant specimens from the archipelago, Darwin nonetheless collected more specimens from more islands than his predecessors. It was these collections that Darwin sent to his botanical mentor John Stevens Henslow, who later passed them on to Joseph Dalton Hooker for study and publication in the first Flora of the Galápagos Islands. In fact, Darwin's collections comprised approximately 75% of Hooker's An enumeration of the plants of the Galápagos Archipelago; with descriptions of those which are new, which was published in 1847. Darwin's collections proved useful not only in the development of his theory on the formation of new species, but also in the relatively young discipline of plant geography.

PRELIMINARY STUDIES OF FLORAL ANATOMY OF physalis (SOLANACEAE). Paige E. Miller & W. John Hayden, Dept. of Biology, Univ. of Richmond, 23173. Near-anthesis flower buds of the tomatillo, *Physalis philadelphica* (syn.: *P. ixocarpa*), and a weedy relative, P. pubescens, were studied via light and scanning electron microscopy using standard techniques. Buds, flowers and fruits of P. philadelphica are larger than those of P. pubescens, but otherwise, the two species are structurally similar. Calyx consists of five fused sepals that are persistent and accrescent through fruit development. The rotate corolla consists of five fused petals each bearing a dark spot near the throat. Uniseriate trichomes, both glandular and eglandular, occur on surfaces of both perianth whorls. There are five stamens; basally, filaments are adnate to the corolla. Filaments are vascularized via a single amphicribral bundle. Anthers are tetrasporangiate, bilocular, and longitudinally dehiscent. The anther wall consists of a persistent epidermis, and a well-marked endothecial layer that varies from one to three cell layers thick; neither middle layers nor tapetum was observed in near-anthesis anther walls. The gynoecium consists of two fused carpels, the ovary region of which encloses two locules and large axile placentas bearing numerous ovules. The thickened base of the ovary wall functions as a nectar-secreting disk/nectary. anatropous and unitegmic. Continuing studies will focus on the floral vascular system, as well as details of anther wall and ovule development.

THE POWER OF GENES IN UNDERSTANDING BIODIVERSITY: THE ROSIDS. Dipan H. Oza, Sunny S. Crawley, Chelsey M. Black & Khidir W. Hilu, Department of Biology, Virginia Tech, Blacksburg, VA 24060. We are assessing here the phylogenetic signal of genomic regions with different modes of evolution in resolving biological diversity using the "rosids" as a case study. The rosids are the largest lineage in flowering plants (angiosperms), comprised of about 70,000 species of diverse biological forms. Molecular phylogenetics brought the rosid families together from traditionally diverse angiosperm subclasses in a rather heterogeneous assemblage. We examine here the phylogenetic signal in two types of genomic regions that differ in rate of nucleotide substitution: 1) three rapidly evolving regions (matK, trnK intron, and matR), and 2) two slowly evolving genes (atpB and rbcL). When trees based on slowly and rapidly evolving genomic regions are compared, both displayed a similar amount of resolution but support for the nodes was significantly higher with the rapidly evolving regions. Using sequence information from all five genomic regions, the

support increases compared to the slowly evolving genomic regions alone, but quite similar to that obtained with the rapidly evolving genomic regions alone. Therefore, this study shows that rapidly evolving genomic regions provide more phylogenetic signal for resolving relationships among the rosids than the traditionally used slowly evolving regions.

HYDROPHILIC AND LIPOPHILIC ANTIOXIDANT CONTENT IN FIVE TROPICAL SPICES. Rachel E. Pence and Michael H. Renfroe, Dept. of Biology, James Madison Univ., Harrisonburg, VA 22807. Dietary sources of antioxidants are important as part of healthy diets because antioxidants are thought to help prevent various chronic diseases and provide multiple health benefits. We analyzed the antioxidant content of five tropical spices: allspice, cinnamon, cloves, ginger and nutmeg. Antioxidant content was measured using the ABTS/H<sub>2</sub>O<sub>2</sub>/HRP decoloration method, and means were compared using a one-way analysis of variance followed by Dunnett's T3 test for significance of differences of means. Cloves had the greatest hydrophilic antioxidant content but the least lipophilic antioxidant content. Lipophilic antioxidant content was greatest in allspice, while the hydrophilic antioxidant content of allspice was second only to cloves. Ginger was relatively low in antioxidants compared with the other spices. Total antioxidants was greatest in cloves. Studies of antioxidant content in spices can provide information helpful to planning healthy diets.

ISOLATION AND CHARACTERIZATION OF LEAF ENDOPHYTES IN BETULA UBER AND BETULA LENTA. Jessica D. Weaver & Kevin G. Jones, Univ. of Virginia's College at Wise, Wise VA 24293. Although originally described as a distinct species, Betula uber is now regarded as a variant of Betula lenta. A ubiquitous characteristic of angiosperms is that their healthy tissues show symptomless internal colonization by fungi called endophytes. The purpose of this research is to initiate a survey of the endophyte complement of Betula uber and Betula lenta and to investigate the effects of leaf shape on endophyte colonization since theses trees differ markedly only in leaf form. Surprisingly, gross morphology of the fungi revealed that there were no common endophytes between Betula uber and Betula lenta. There is great diversity in endophyte populations within and between these species; but, our results may simply reflect the natural diversity of endophytes. Because there is such diversity, no conclusions about the effects of leaf shape can be made at this time, and more samples need to be collected and analyzed. (Supported by the Virginia Academy of Science).

### Chemistry

THE ENZYMATIC ACTIVITY OF MshA: A FUNDAMENTAL ENZYME IN MYCOTHIOL BIOSYNTHESIS. T. W. Boshers & M. Hernick, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. Mycothiol is the primary reducing agent used by mycobacteria to prevent against oxidative damage. Consequently, enzymes involved in mycothiol biosynthesis are targets for antibiotic development. MshA is a glycosyltransferase that catalyzes the transfer of GlcNAc from UDP-GlcNAc to

inositol-1-phosphate to form GlcNAc-Ins-1P and UDP, a key step in the biosynthesis of mycothiol. We are working towards the biochemical characterization of recombinant MshA from *M. smegmatis* and *M. tuberculosis*. MsMshA has been expressed in *E. coli* and purified using amylose resin. We have developed an HPLC-based assay to measure MshA activity, and have used this assay to demonstrate that the recombinant MshA from *M. smegmatis* is active. We will use this assay in the future to characterize the chemical mechanism of MshA.

HYDROXAMATE FORMATION IN SIDEROPHORE BIOSYNTHESIS. Chocklett & P. Sobrado, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The proliferation of many microorganisms depends heavily on their ability to scavenge nutrients from their host and extracellular spaces. Since iron is an essential nutrient, microbes have developed a tightly regulated system to sequester iron and utilize this element for its metabolic needs. Like many other pathogens, M. tuberculosis, P. aeruginosa, B. cepacia and A. fumigatus synthesize and secrete low molecular weight (M<sub>r</sub> < 2000) Fe<sup>III</sup> chelators called siderophores under iron-limiting conditions. Pathogens then specifically take up the siderophores complexed with ferric iron, thereby increasing the concentration of iron to levels necessary for pathogens to proliferate during infection. The assembly-line process of siderophores has revealed that a critical step in siderophore biosynthesis involves the hydroxylation of the terminal amino group from an amino acid by a flavin-dependent monooxygenase at the expense of NAD(P)H and O<sub>2</sub>. The hydroxylated product from this reaction is then incorporated into the backbone of the siderophore, where it directly coordinates ferric iron. Here, we report the characterization of two enzymes catalyzing the initial step in siderophore biosynthesis in the prokaryote Mycobacterium smegmatis, as well as the fungus Aspergillus fumigatus. This is the first report of members of the NMOs containing a bound FAD cofactor upon purification, assisting in the detailed biochemical characterization of this class of flavoprotein monooxygenases.

METHOD DEVELOPMENT FOR THE DETERMINATION OF AMINO ACID IN SEXUALLY MATURE Odocoileus virginianus BY GAS CHROMATOGRAPHY MASS SPECTROMETRY. S. H. Clift, J. N. Watson, D. Swale, M. Houck, L. S. Webb, & G. C. Klein, Dept. Of Biology, Chemistry and Environmental Science, Christopher Newport University, Newport News VA 23606. The purpose of this study is to develop a method that can be used to quantify the amino acids present in a sample of muscle tissue from Odocoileus virginianus using Gas Chromatography Mass Spectrometry (GC-MS). Because amino acids are multifunctional, they are difficult to analyze by a single technique; however, they can be derivatized using N-tertbutyldimethylsilyl-N-methyltrifluroacetemide (MTBSTFA). This derivatizing agent attaches to amine nitrogens and carboxylate oxygen sites on the amino acids, and create suitably volatile compounds for use with GC-MS. Fragmentation occurs at similar cleavage sites for all amino acids, allowing for quick and easy identification of each of the separated peaks in the gas chromatogram. Currently, data are being collected for derivatized standard amino acids at different concentrations in order to provide calibration data for the application of this method. These techniques will be used to quantify amino acids extracted from the muscle tissue of sexually mature *Odocoileus virginianus*.

COMPETITION BETWEEN SUBSTITUTION AND ELIMINATION IN THE REACTIONS OF DIANIONS WITH SUBSTITUTED AND CYCLIC ALKYL HALIDES. Keyanna Conner, Renan Joviliano, Andrew Alexander & Scott Gronert, Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284-The competition between substitution and elimination in the nucleophilic reactions of alkyl halides is an important testing ground for reactivity patterns in gasphase organic chemistry. The dianions in the study employ sulfonates as inert, spectator ionic sites and phenolates or benzoates as the nucleophilic ionic sites. They were generated in the gas-phase by ESI in an LCQ ion trap mass spectrometer. Using a custom-built interface, alkyl chlorides or bromides were introduced into the helium buffer gas of the instrument. Reactions were monitored as a function of time at various flow rates (pressures) of the reagent gases and branching ratios were determined. Reactions were modeled computationally at the MP2/6-31+G\*\* level and the results aid in the interpretation of the experimental data. Three groups of alkyl halides were examined. Alkyl chlorides with electron-withdrawing groups at the beta position produced strong activation of the elimination pathway. Investigations using cyclic alkyl bromides indicated the balance between substitution and elimination are sensitive to ring size with a large shift from substitution to elimination in the move from cyclopentyl to cyclohexyl bromide. A series of six bromopentanes produced widely varying substitution patterns at both the alpha and beta carbons.

DENSITY FUNCTIONAL THEORY STUDIES OF DIARYLAMINOSTYRYL CHROMOPHORES WITH HETEROCYCLIC AND VINYLENE BRIDGES. Courtney E. Dula & Edmund Moses N. Ndip, Chemistry Department, Hampton University, Hampton, VA 23668. Organic systems with large 2PA cross-sections have a broad spectrum of technological applications, i.e. Organic Light Emitting Diodes. Organic semiconductors, like the ones studied, show strong nonlinear optical properties due to the presence of delocalized electrons in the  $\pi \to \pi^*$  orbitals. These materials are ideal for fast processing of information and optical data storage applications. The linear absorption spectra, energy gaps, intensities and/or oscillator strengths, dipole moments, and other molecular properties have been computed at semi empirical and ab initio levels of theory. The computed  $\lambda_{max}$  for the thiophene compounds (423-437nm) are in good agreement with the experimental data (405-438nm). Additional calculations have been carried out for analogous compounds based on the success of the previous calculations. These are the first quantum mechanical studies that have been performed on these types of thiophene, furan, and pyrrole heterocyclic centrosymmetric systems. The data show reasonable agreement between Arguslab (ZINDO-CI) (398-420nm) and TITAN (DFT: B3LYP - 6-31G\*\*) (421-512nm)  $\lambda_{max}$ . However, the band gaps that were extracted from the B3LYP calculations (1.16 - 2.02eV) are more consistent with experimental values (2.81-3.07eV) for the thiophene compounds.

RELATIVE STABILITIES OF C<sub>94</sub> AND C<sub>94</sub>. IONS USING QUANTUM AND STATISTICAL MECHANICAL FUNCTIONS. <u>Tim Fuhrer</u>, & Harry C. Dorn,

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061.  $C_{94}$  is one of many larger cage fullerenes being studied today. Previous experiments and calculations have found the  $C_{3v}$  symmetry empty cage for  $C_{94}$  to be unstable, but when  $M@C_{94}$  (M=Ca, Tm) was synthesized in our lab and an independent lab in China, only the  $C_{3v}$  isomer is found. Quantum and statistical mechanical calculations show that the  $C_{3v}$  cage may be stabilized by the addition of the two electrons donated by the metal atom while the other more stable empty cage isomers are destabilized by the addition of those two electrons.

OXIDATION OF ALCOHOLS USING ALUMINA BOUND PCC UNDER HETERO-GENEOUS REACTION CONDITIONS, Kristen Jobes, Heather Robinson, & Emma Goldman, Department Of Chemistry, University of. Richmond, Richmond VA 23173. Our research project involves studying heterogeneous high temperature (100-200?C) gas/solid reactions. Previously substitution and elimination reactions using metal alkoxides have been studied. Our recent focus is specifically on oxidizing alcohols with PCC under the heterogeneous high temperature gas/solid reaction conditions. The successful oxidation of these alcohols provides data to support heterogeneous gas/solid reactions as a viable alternative reaction method. The adoption of the heterogeneous method in laboratories will reduce the wasteful use of solvents and help laboratories move towards the goals of Green Chemistry.

METHOD DEVELOPMENT FOR THE QUALITATIVE DETERMINATION OF PETRO-PORPHYRINS OF TWO GEOGRAPHICALLY DISTINCT CRUDE OILS BY UV-VIS SPECTROSCOPY. G.C. Klein, K.J. Anderson, R.G. Saller, & M.A. Beasley, Dept. Of Biology, Chemistry and Environmental Science, Christopher Newport University, Newport News VA 23606. Petroporphyrins are metal-containing biomarkers found in crude oils that are known to deactivate catalysts during the refining process. Quantification of petroporphyrins in crude oils will lead to efficient removal procedures increasing the overall production of petroleum products. Fractionation schemes were investigated to increase the overall recovery yield of the heavy crude oil. UV-Vis Spectrophotometry was used to screen each of the fractions for the presence of petroporphyrins. The use of the continuum-removal by division mathematical model assisted in the identification of these petroporphyrins by increasing the definition of the associated peaks. Petroporphyrins were found in 4 of the 6 fractions of our separation scheme. UV-Vis Spectrophotometry can be used not only as a means to screen fractions for the presence of petroporphyrins, but also can be used to potentially provide information on the type of crude oil (light vs. heavy).

POST-TRANSLATIONAL MODIFICATIONS OF MODEL PROTEINS WITH 4-HYDROX-YNONENAL, A QUANTITATIVE ANALYSIS OF REACTIVITY AT SPECIFIC SITES. Qingyuan Liu and Scott Gronert, Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23284-2006. HSA was incubated with 4-hydroxynonenal (HNE) in PBS at molar ratios (HNE:HSA) ranging from 5:1 to 100:1 from 1 to 24 hours. The adducts were stabilized with sodium borohydride, digested with trypsin. The tryptic peptide mixtures were labeled with one of four iTRAQ reagents and then combined with other labeled samples. We performed two general

types of experiments to probe the kinetics of the reaction system. In the first, varying ratios of HNE/HSA were employed with a fixed reaction time of 3 hours. In the second, a high ratio (100:1) was used and measurements were taken at several times. The latter allowed us to probe slower processes. Kinetic data were obtained for 17 modification processes. The one free cysteine in HSA is highly reactive and in general, the histidines are more reactive than lysines. However, there is a large variation in reactivity among the histidines in HSA. Aside from the free cysteine, we find the following order of reactivity in the more reactive sites: His<sup>312</sup> > His<sup>534</sup> > Lys<sup>549</sup> > His <sup>91</sup> > His<sup>266</sup>/His<sup>271</sup>> Lys<sup>375</sup> > His<sup>391</sup> (Swiss-Prot). The results suggest that reactions occur on multiple time scales, possibly including protein conformational changes, and this is a likely cause for the differences in previous reports.

A COMPARATIVE STUDY OF THE NON-LINEAR OPTICAL PROPERTIES OF A SERIES OF PUSH-PULL DIARYLAMINOSTYRYL CHROMOPHORES WITH HETEROCYCLIC VINYLENE AND AZO BRIDGES. Edmund Moses, N. Ndip & Courtney E. Dula, Chemistry Department, Hampton University, Hampton, VA 23668. In the present study, a number of molecular descriptors (linear absorptions, dipole moments, transition dipoles, hyperpolarizabilities, etc.) have been computed for several series of diarylaminostyryl heterocyclic (thiophene, furan, and pyrrole-based ) chromophores with vinylene and azo bridges at the semi-empirical (Arguslab ZINDO-CI) and ab initio (DFT/B3LYP using Gaussian 98, Spartan 06, and Titan) molecular orbital methods. The influences of terminal substituents on various molecular properties of the homologues were also examined. The HOMO/LUMO surface renderings show a greater population in the HOMO orbital stretching toward the terminal substituents in the azo compounds, the vinylene compounds show a decrease in the size orbitals on the benzene ring. The LUMO for the vinylene compounds show an increased population of the lobes around the benzene, whereas in the azo compounds the sizes of the lobes have decreased. When azo bridges were substituted for the vinylene bridges in the compounds an increase in the absorption maxima were observed (vinylene 421-512nm, azo 485-528nm). The increased electron density and electrostatic potential from the azo bridge suggests that the bridge substitution from vinylene to azo improves the conduction (exhibit stronger NLO properties) properties of these materials.

CHARACTERIZATION OF Aspergillus fumigatus UDP-GALACTOPYRANOSE MUTASE. Michelle Oppenheimer and Pablo Sobrado, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. UDP-galactopyranose mutase (UGM) catalyzes the conversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf). UDP-Galf is then used for the addition of galactofuranose to the cell wall of Aspergillus fumigatus, where it plays a key structural role. Deletion of A. fumigatus UGM (AfUGM) results in attenuated virulence. In addition, Galf is absent in humans therefore AfUGM is considered an ideal therapeutic drug target for the treatment of aspergillosis. UGMs are also unique because of their novel chemical mechanism, in which the flavin must be reduced yet no net redox change occurs throughout the reaction. Studying the mechanism, as well as, the structure of AfUGM will provide valuable knowledge which can be used for the generation of new drugs for the

treatment of aspergillosis. Currently, we have been able to successfully recombinantly express and purify, crystallize, and initially characterize AfUGM, setting the stage for the future mechanistic studies and development of therapeutic drugs.

DIASTEREOSELECTIVE  $\alpha$ -ALKYLATION OF CHIRAL  $\beta$ -BORYLATED ESTERS. Michael Perfetti & Webster Santos, Dept. of Chemistry, Virginia Tech, Blacksburg, VA 24061. The use of boron in the development of asymmetric methodologies and inhibitor synthesis has increased significantly over the last decade. Currently, the use of chiral boronic esters to enhance the stereochemistry of molecules through intramolecular diastereoselective alkylations is absent in the literature. We report the development and initial characterization of a novel intramolecular diastereoselective reaction for the α-alkylation of chiral β-borylated esters. We propose that standard deprotonation of chiral β-borylated esters with lithium diisopropylamide (LDA) leads to the formation of an intramolecular cyclic five-membered ring boron-"ate" complex. Upon treatment with an alkylation reagent, this complex will collapse to provide chiral  $\alpha$ ,  $\beta$ -substituted boronic esters with a high degree of diastereoselectivity. This reaction is powerful in that a wide range of chiral β-borylated ester substrates can be employed that possess varying degrees of substitution and steric bulk. Results show that yields up to 67% are achievable with diastereomeric ratios (DR) of (9.7:1), where alkylation products from bulkier tert-butyl esters provided higher DR values compared to methyl esters that possessed the same β-functional groups. Additionally, several <sup>11</sup>B NMR techniques were used to investigate and elucidate the mechanism of this reaction.

GROUP 5 BACTERIAL MULTICOMPONENT MONOOXYGENASES. Robinson, M. Oppenheimer, J. Llanos-Velázquez, S.W. Chocklett & Sobrado, P., Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. Microbes that contain multicomponent monoxygenase systems can use organic compounds such as tetrahydrofuran (THF) or propane as the sole carbon and energy source. The ability to metabolize these compounds is related to the activity of the Group V Bacterial Multicomponent Monooxygenases (BMMs). Members of the Group V BMMs share the same operon orientation and conserved components, which consist of: i) a hydroxylase with an  $\alpha$  catalytic subunit and a  $\beta$  structural subunit, ii) an oxidoreductase, and iii) a regulatory subunit to prevent futile cycling. The oxidoreductase is responsible for transferring electrons from NADH, through its FAD and 2Fe-2S cluster, to the diiron center of the hydroxylase. The reduced hydroxylase then reacts with molecular oxygen leading to the formation of a high-valent iron-oxo species, which is required for the hydroxylation of the substrate. We present the results of the cloning, expression, purification, and biochemical characterization of the recombinant forms of ThmD and PrmC from Pseudonocardia sp. Strain K1 and Gordonia sp. Strain TY-5. These are the oxidoreductase components of the thm and prm operons, for which the operon has been isolated from these organisms. Also reported is the expression, purification and crystallographic studies of ThmC and PrmD, the regulatory domains of each operon, respectively

MODIFICATION OF MACROPOROUS SILICA THIN FILMS VIA METAL NANO-PARTICLE DEPOSITION. Amy E. Rue & Maryanne M. Collinson, Dept. Of

Chemistry, Virginia Commonwealth University, Richmond VA 23284-2006. Two methods were used to deposit metal nanoparticles into the cavities of sol-gel derived silicate thin films created from polystyrene sphere templates. The first method followed a bottom up electrochemical approach, where films were formed on glassy carbon electrodes from a 0.4 µm polystyrene sphere doped sol prepared from the condensation of tetramethoxysilane (TMOS). Since pinholes in the film were filled by electrodeposition of TMOS, the only available surface for electrochemistry was at the bottom of the cavities. Copper was then electrodeposited into the exposed cavities. Changes in the deposition length resulted in variation in particle size. The second method used a top down approach for the electrolessdeposition of gold. Similar films were formed on glass, and the deposition occurred as a result of the attraction between prepared gold nanoparticles and a monolayer of (3-Mercaptopropyl)trimethoxysilane. To insure the placement of the gold only occurred in the cavities, the exposed film was blocked with octyltrimethoxysilane or trimethylchlorosilane before template removal. Films were characterized using atomic force microscopy. Measurements were taken before and after deposition, and showed the presence of copper particles ranging from 100 to 300 nm and gold particles ranging from 70 to 100 nm.

**SUBUNIT** INTERACTION CLONED HUMAN OF THE **GUANINE-7-**METHYLTRANSFERASE. Jessica N. Skeeter, Jeanhee Chung & Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The 5'-end of eukaryotic mRNA is capped and the guanine base is methylated in the N-7-position generating a fully functional cap structure. If the cap is not methylated at the N-7 position, the mRNA is not translated. The enzyme that methylates the N-7 position, Guanine-7-methyltransferase, has been expressed as a His-tag protein in E. coli. and purified on a Nickel column. The full length enzyme, 476 amino acids long, and the deletion mutation, 120 amino acids removed from the N-terminus are about 80% pure after the nickel column. To further purify the enzymes, a positively charged ionexchange column (Mono Q-Sepharose) was used. The elutants from the Nickel column were applied to Mono-Q columns and eluted with increasing concentrations of KCl. A major methyltransferase peak eluted at 0.1 and 0.15 M KCl and was over 95% pure as determined by SDS polyacrylamide electrophoresis. This purified guanine-7methyltransferase was then applied to a FPLC-Superose 12 gel exclusion column to characterize the subunit structure. Two major peaks of protein were observed for both the full length and the deletion mutation enzyme which corresponded to about 90% homodimer and 10% monomer for each respectively. The purified enzymes were also analyzed by blue-native polyacrylamide gel electrophoresis and the deletion of 120 amino acids had no affect on the subunit interaction, i.e. about 90% dimer

IR AND NMR OF ALCOHOLS IN THE VAPOR PHASE: A COMPARISON BETWEEN EXPERIMENTAL RESULTS AND DENSITY FUNCTIONAL CALCULATIONS. C.C. White & T.C. DeVore, Dept of Chem. and Biochem., MSC 4501, James Madison University, Harrisonburg VA 22807. The observed IR and NMR spectra of the methanol, ethanol, 2-propanol and 2-methyl, 2- propanol (tbutanol) vapor molecules are compared to the spectra calculated for these molecules using the DFT-B3LYP method with the 6-311++G (3df, 3pd) basis set using the

Gaussian 03 software. NMR spectra were calculated using the GIAO method. IR spectra (4000 to 400 cm<sup>-1</sup> at 0.5 cm<sup>-1</sup> resolution) were obtained by injecting 10 µL of the alcohol into a 10 cm pathlength stainless steel vacuum cell place in the sample compartment of a Nicolet 6700 FTIR. Vapor phase NMR spectra were obtained by placing 0.15 mL of the alcohol in the outer tube of a standard 5 mm OD double NMR tube. A Bruker Spectrospin 400 and a Bruker Spectrospin 600 NMR equipped with a variable temperature probe were used to collect the NMR spectra. D<sub>2</sub>O was placed in the inner tube as the lock solvent. While the calculated chemical shifts were in qualitative agreement, the quantitative values varied by ± 0.2 ppm when adjusted to the same reference. The IR frequencies showed a decreasing error at the lower frequency. Since the calculations give the harmonic frequency, part of this difference may result from ignoring the anharmonic terms. The relative intensities generally agreed within a factor of 2.

SIZE TUNABLE HIERARCHICAL POROUS STRUCTURES BY DIRECT TEMPLATING. <u>Bo Zhao</u>, & Maryanne Collinson, Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23284-2006. Materials with bimodal porosity are of great interest in a diversity of applications of separation, catalysis, and sensing system. In this work, hierarchical porous materials were made by colloidal crystal templating with "raspberry" and "strawberry" shaped templates. These monodispersed hierarchical templates were prepared by simply coupling polystyrene beads of different sizes together. Both the "center" and "satellite" spheres can be varied, and the coverage of "satellite" spheres on the surface of the "center" sphere is tunable by reducing the coupling speed. Based on the templates, bimodal hierarchical porous silica monoliths and gold films were fabricated by sol-gel templating method and electrodeposition, respectively. The materials possess adjustable and well-defined bimodal pore sizes with interconnected windows. In this presentation, the synthesis and applications of these unique materials will be discussed.

## **Computer Science**

USING SECOND LIFE FOR COMPUTER SCIENCE EDUCATION. Robert A. Willis Jr. Department of Computer Science, Hampton University. Over the past few years, I have noticed that our students are reluctant to approach learning computer science in the traditional ways. Computer science requires beginning students to learn the concepts of computer science and the art of programming. While disparate, both of these facets require a good deal of study using texts and practice. Second Life is used to implement a number of innovative interactive tutorials tailored for this generation of students. Furthermore, the environment is conducive for instruction in a number of other areas in computer science (and other disciplines). Second Life is a three dimensional virtual world. It is a social environment that allows people to "live" much as they do in real life. People (represented as avatars) can purchase land, build houses, work, play, and participate in many other activities. It is an ideal environment to reach all levels of students.

INTERACTIVE PARAPHRASE TRAINING: THE DEVELOPMENT AND TESTING OF AN ISTART MODULE. Chutima Boonthum. Department of Computer Science, Hampton University. Hampton, VA. Comprehension of science texts is challenging, particularly when the reader lacks the skills or knowledge necessary to fill in conceptual gaps in the text content. The iSTART system was developed to help readers learn and practice reading strategies to improve their ability to comprehend challenging text. This study describes a new iSTART module recently developed and tested, called Interactive Paraphrasing (IP), in which students are interactively and adaptively taught how to paraphrase sentences. We compared the effects of iSTART to iSTART with IP (IP-iSTART) with high school students on their strategy use and ability to comprehend text. IP-iSTART increased skilled readers' self-explanation quality, improved their ability to answer online comprehension questions, and increased their use of paraphrases after training. Less skilled readers benefited most in self-explanation quality from the original version of iSTART. Results are discussed in terms of tailoring reading strategy training to the needs of the reader.

GENERATION Y AND COMPUTER LITERACY/EDUCATION. Angela Hayden. Department of Computer Science, Hampton University Hampton, VA. The generation of Americans born between 1977 and 1994 are affectionately known as Generation Y. They hold to similar values of their parents, but will challenge authority and the information given them in any setting. They possess a variety of skills including computer skills, making them the most computer literate of all generations prior to them. They can be stimulated through a variety of means, most of which are visual and audio. They also appreciate having fun more than just learning facts. Strategies for both study and pedagogy offered as suggested means to help students learn have not changed much in recent years and can still be used for those entering college over the next two or three years. One such strategy includes visual/auditory where students are asked to read aloud, record and play back definitions to terms, or visualize certain tasks. At HU, we offer students in our CSC 120, Intro to Computer Literacy course a method that requires them to do much more than just passively sit in class and take notes. This method, where students learn computer applications using hands-on activities, is not without its problems and challenges, but overall most students do extremely well and some have express not only satisfaction with the course, but acknowledge that learning has occurred.

### **Education**

INCORPORATING LEARNING STYLES INTO A SCIENCE LECTURE COURSE. Lisa S. Webb, Christopher Newport University, Department of Biology, Chemistry and Environmental Science, 1 University Place, Newport News VA 23606. Learning is a complex and highly individualized process that can, and does, occur in a variety of modalities. Students can learn visually, through pictures, diagrams, charts, animations and reading; they can learn aurally, through listening to lectures, discussions, music and conversations; they can learn in a tactile manner, through manipulation of a three-dimensional model or tracing the shape of a graph with their fingers; and they can learn

in any combination of these, or other, ways. Because teaching and learning are complementary processes, effective instruction must occur via multiple pathways and incorporate multiple modalities, the choice of which should be driven by student learning preferences. In my lecture courses, I ask students to take a free, online assessment of their learning styles (www.vark-learn.com) and turn in the results for a quiz grade. This informs students of their own learning style(s) and preference(s) and gives them the opportunity to develop strategies to exploit these in preparing for class and studying for exams. It also informs me, the instructor, of the learning preferences of the students, which allows me to plan instructional strategies intended to actively and effectively engage my students in the process of learning.

APPLICATION OF THE INTERACTIONAL MODEL OF CULTURAL DIVERSITY TO IDENTIFY DIVERSITY CLIMATE FACTORS ASSOCIATED WITH ORGANIZATIONAL EFFECTIVENESS IN ACCREDITED U.S. PHYSICAL THERAPIST EDUCATION PROGRAMS. Elizabeth F. Giles, School of Physical Therapy, Old Dominion University, Norfolk, VA 23529. This work evaluated the effectiveness of the Interactional Model of Cultural Diversity as a theoretical framework to identify diversity climate factors associated with organizational effectiveness in accredited U.S. physical therapist education programs (N=151; RR=83.9%). A descriptive cross-sectional research design examined two model constructs. Cronbach's alpha coefficients were .82 for the IAPCC-R and .78 for the perception of diversity climate scale adapted from The Diversity Survey. Only 43% of all study programs reported excellent diversity climates. Pearson chi-square results (alpha=0.05) showed statistically significant construct relationships. A Kruskal-Wallis test and a post-hoc analysis determined statistically significant program group differences in minority graduates based on faculty diversity. Perception of diversity climate scale score was a significant predictor of number of minority graduates and percent minority graduates. Multiple logistic regression models were significant for predictors of number of graduates (p=.000; Nagelkerke's R<sup>2</sup>=.336), number of minority graduates (p=.000; Nagelkerke's R<sup>2</sup>=.534; .334), and percent minority graduates (p=.000; Nagelkerke's R<sup>2</sup>=.562; .347). The Interactional Model of Cultural Diversity was effective in modeling these construct relationships in accredited U.S. physical therapist education programs.

RE-CREATING A SCIENCE MUSEUM FOR THE 21<sup>ST</sup> CENTURY. Richard Conti, Science Museum of Virginia, Richmond, VA. The Science Museum of Virginia is the Commonwealth of Virginia's flagship institution for informal science learning. Established in the early 1970s, the museum is undergoing a process to reinvent itself for the 21<sup>st</sup> century. For the past six months, the museum has undergone an extensive process to engage its constituents and reinvent itself to become a more contemporary, dynamic and relevant institution. Challenges to this process include reaching the entire state with limited resources, managing a network of satellite museums and competing with numerous entities for the discretionary time of our audiences.

CONJECTURAL INDUCTIVISM AND MATHEMATICAL PROOF. W. Michael Gentry, Department of Mathematics, Mary Baldwin College, Staunton, Va 24401.

Given sufficient time and incentives students can and will discover that none of the integers 11, 111, 1111, 1111, etc. is a perfect square. Using modulo 4 arithmetic, this conjecture is not ifficult to prove making it a theorem, indubitably true forever. Not all conjectures are created equal. Students are also able to provide copious inductive evidence that combining the processes of multiplying any odd positive integer by 3 and adding 1, and dividing any even positive integer by 2, always seems to dead end in the sequence 1, 2, 4, 1, 2, 4, 1, 2, 4, 1, 2, 4, etc. However, this conjecture, despite the best efforts of the mathematical community, has not been proven. Is there a pedagogical approach that lends itself to helping students in substantive ways to follow the yellow brick road to provable conjectures, and not venture off into the Land of the Giant of Despair where dragons and nearly unprovable conjectures lurk?

#### **Environmental Science**

THE EFFECTIVENESS OF WETLAND MITRIGATION BANKS IN THE LOWER RAPPAHANNOCK WASTERSHED THROUGH MAPPING AND CREATION OF A SINGLE DATABASE. Brittany A. Baker, Michael L. Bass, Earth and Environmental Sciences, University of Mary Washington, Fredericksburg, VA. Wetlands are a precious environmental resource that provide habitat, prevent pollutants and excess sediments from entering large water ways, and control storm surge. Wetlands may be destroyed in the process of commercial, residential and infrastructure development. Federal regulation requires that these wetland losses must be mitigation, through wetland mitigation banking. Wetlands mitigation banking creates a large area of wetland acreage where portions, measured in credits, are sold to those who have destroyed wetland areas. In the United States Army corps of Engineer's Norfolk district, there are several wetland mitigation banks. Information about each of these banks may be obtained on their RIBITS database. On this database, however, it is difficult to compare attributes of different wetland mitigation banks within the same service watershed. The purpose of this study is to explore the effectiveness of the wetland mitigation banks that service the Lower Rappahannock Watershed by creating a single database for easy comparison and visualizations that may drive future wetland mitigation bank development decisions.

IMPACTS ON TWO STREAMS CAUSED BY DEVELOPMENT IN THE CELEBRATE VIRGINIA NORTH PROJECT. Katherine Vrobel, Earth and Environmental Science Department, University of Mary Washington, Fredericksburg, VA. This study observed and assessed damage to streams located within the Celebrate Virginia North development in Stafford County, Virginia. Research was conducted at seven stations located on England run and the Unnamed Tributary streams. Assessments were made based on the study of the macrobenthic communities, water chemistry comparisons (such as nutrients, dissolved oxygen, conductivity, alkalinity, and water hardness) before and after rainfall, suspended load in the stream water, and gain size analysis in the water column and sediment. This study consisted of a number of methods: Water quality was assessed by determining the abundance and diversity of macrobenthic organisms, which included the Hilsenhoff Family Biotic Index (HBI) and the total percent of insect orders Ephemeroptera, Plecoptera, and Trichoptera

(%EPT) in the biotic community. Water chemistry date, such as dissolved oxygen, conductivity, and temperature, were determined using the YSI Model 85 multimeter. Samples of streams water were collected to determine nutrient levels, alkalinity, hardness, and particle size; particle size; particle size was ascertained with a Coulter counter, and water quality was assessed using LaMotte chemistry kits. Total dissolved solids and total suspended solids were determined by filtering samples from each stream before and after rain, and a gain size analysis was conducted on sand that had accumulated in Stream 5's bed. Results indicate that stream quality is declining rapidly due to the large influx of sediment from the nearby development, which is adversely affecting water quality and smothering macrobenthic communities. A negative relationship is present between the embeddeness in the macrobenthic habitat caused by sediment erosion from the development activities and the health of the streams' macrobenthic communities.

MONITORING THE STORMWATE MANAGEMENT PONDS OF CENTRAL PARK AND DEVELOPMENT OF AN OFFSITE WETLAND MITIGATION PROGRESS. Katherine Oldham and Michael L. Bass, Earth and Environmental Science, University of Mary Washington, Fredericksburg, VA. When Silver Company built the commercial complex of Central Park in the 1990's, 6.9 acres of the wetlands were destroyed. The EPA requires that the equivalent area of destroyed wetland has to be reconstructed. In compliance with the Clean Water Act, wetlands were constructed in two areas; benches around the storm water management ponds located within Central Park, and an off-site constructed wetland in Spotsylvania County. The off-site wetland was impacted by nearby developments. The storm water management ponds receive runoff from Central Park while the off-site wetland receives runoff from a new housing development. Water chemistry tests were performed on both sites, testing for nitrate, phosphate, total alkalinity, total hardness and pH levels. Temperature, dissolved oxygen, and conductivity were measured with an YSI multi meter. The nitrate, phosphate, total alkalinity and total hardness were performed in the lab using LaMotte testing kits. Samples were taken before major rainfall as well as after storm events in order to examine the impact of runoff from the development. Identical water chemistry tests were performed on the off-site constructed wetland as well as a nearby natural wetland. In addition to water chemistry tests, a survey of woody stems was done on the off-site wetland in order to determine the number of woody stems per acre. The wetland was divided into two sections, and each section was marked off in 100 foot increments with string. 20 foot squares were constructed on either side of the 100 foot markers to create a grid. Within each square the number of woody stems over one foot in height were counted and classified. In addition, wetland professional Bill Sipple aided in identifying herbaceous plant species and creating a list of those species. Soil cores were taken along the 100 foot markers in order to assess the hydric soil prevalence which is substantial.

COMPARING CLIMATE RESPONSES IN TWO TREE SPECIES OF MOUNT VERNON, VA. Brittany Miller & Daniel L. Druckenbrod, Biological and Environmental Sciences Dept., Longwood University, Farmville, VA 23909. This research investigates the relationship between tree growth and precipitation for two

common long lived species in the eastern deciduous forest. Using tree ring cores of select Mount Vernon Virginia pine (*Pinus virginiana*) and white oak (*Quercus alba*) collected in June 2008, we crossdated annual ringwidths with divisional climate data overlapping back to the year 1895. Significant correlations were found between precipitation and tree ring growth using COFECHA, ARSTAN, and DendroClim 2002 programs in conjunction with precipitation data from the National Oceanic and Atmospheric Administration. It was determined that June's precipitation was most important to Oak growth, and May and prior September precipitation was most important to Pines.

FOREST COVER CHANGE OF HISTORICAL MOUNT VERNON FROM 1793 TO 1994. Heather M. Carty & Daniel L. Druckenbrod, Biological and Environmental Sciences Dept., Longwood University, Farmville, VA 23909. During European settlement the forests were heavily logged and farmed, stripping the majority of all the forest lands in eastern North America (Foster and Motzkin 1998). However, by the mid 19th century with the rise of the Industrial Revolution, the decline of agriculture, and forest use the forests of North America have increased in area and in age (Foster and Motzkin 1998). This means that currently in the United States there is more forest cover than there has been since European settlement. Although this may be the case for the entire eastern seaboard, this study hypothesizes that the current forest cover of Mount Vernon is smaller than during George Washington's era due to human impact and development. The conclusions from this research support the hypothesis. The forest cover of Historical Mount Vernon has decreased since Washington's era. This GIS project uses a survey of Mount Vernon drawn by George Washington, a Civil War topography map, a 1933 topography map, and current aerial photos of Mount Vernon. All illustrate forest cover for its time period and use the Universal Transverse Mercator NAD 1983 coordinate system (zone 18). The two historical maps and the 1933 topography map were georeferenced against the aerial photos. George Washington's hand drawn map is the most accurate map that was georeferenced to the aerial photo, the 1933 topography map was the second most accurate, and the Civil War Map had the largest error. After overlaying all three maps with the aerial photo only 44 Acres of Washington's original 2,300 Acres of forest land remains consistently forested.

A PROPOSAL TO ESTABLISH A NATIONAL MUSEUM OF ENVIRONMENTAL SCIENCE. Richard S. Groover, J. Sargent Reynolds Community College, Richmond, VA. It is proposed that a National Museum of Environmental Science be established at a four-year institution in Virginia. Such a museum should include a small public exhibit on environmental science issues, an environmental persons Hall-of-Fame, a collection of first edition books on the science of the environment, up-to-date reports on environmental conditions, working documents from those persons who advanced the science and concerns about the environmental issues, artifacts from early environmental movement activities, and audio-visual interviews with early pioneers of the environmental movement and environmental science. The Museum physical plant would include a library, storage space for artifacts and documents, staff office space and a meeting facility for symposia.

BASELINE WATER QUALITY ASSESSMENT USING MACROINVERTEBRATES IN HIATT AND LICK RUNS, OPEQUON CREEK WATERSHED, FREDERICK COUNTY, VIRGINIA. Marie R. Dahl<sup>1</sup>, Jared B. Davis<sup>1</sup>, Andrew G.M. Fisher<sup>1</sup>, L. Brandon Millholland<sup>1</sup>, J.W. Pangle<sup>2</sup>, Sean G. Robertson<sup>1</sup>, Jeremy D. Tovar<sup>1</sup>, & Woodward S. Bousquet<sup>1</sup>, <sup>1</sup>Environmental Studies Department, Shenandoah University, Winchester, VA 22601 and <sup>2</sup>Opequon Creek Targeted Watershed Grant. In 1996, the Virginia Department of Environmental Quality placed Opequon Creek in Frederick and Clarke Counties on its Impaired Waters List because it failed to meet water quality standards for aquatic life and E. coli bacteria. Shenandoah University student researchers sampled benthic macroinvertebrates (BMIs) at six locations in the Hiatt-Lick Run subwatershed of Opequon Creek in May and October 2008. Methods and analysis conformed to the EPA's Rapid Bioassessment Protocols (RBPs) and the Virginia Stream Condition Index (VSCI) manuals, respectively. The VSCI is an eight-metric index based on the biodiversity, pollution tolerance and ecological niches of the BMIs collected in each sample. The mean VSCI score for the 11 samples was 43.5 on a 100-point scale, an overall water quality rating of "moderately stressed". VSCI scores for 8 samples fell into the severely or moderately stressed category, 2 were rated fair, while only 1 was rated good. This study provides provide baseline data and a sampling framework to evaluate proposed watershed improvements under the TMDL (Total Maximum Daily Load) Implementation Plan for Opequon Creek.

LOST CRAB-LOST CULTURE: ENVIRONMENTAL AND CULTURAL CHANGES RELATED TO THE FRESHWATER CRAB, POTAMON IN GREECE. Eugene G. Maurakis<sup>1,2,3</sup> and David V. Grimes. <sup>3</sup> Science Museum of Virginia, 2500 W. Broad St., Richmond, VA 24542, <sup>2</sup>Biology Dept., University of Richmond, VA 23173, <sup>3</sup>VA Dept. of Environmental Quality, Richmond, VA 23060. Objectives are generate phylogenetic relationships and biogeographic hypotheses of four freshwater crab species of Potamon in Greece; and comment on the need to protect their habitat. Potamon in the Balkan peninsula and islands in the Mediterranean region is a monophyletic group composed of two main clades: Clade 1 (P. fluviatile and Potamon algeriense) and Clade 2 (P. ibericum and its sister group composed of Potamon rhodium and Potamon potamios). Vicariant events (e.g. transgression and regression, orogeny, volcanism) are hypothesized as major factors shaping distributions of Potamon species in the region. We recommend an increase in environmental education and communication among older and younger generations, agriculturalists, politicians, policy writers, land developers and economists to create an understanding for the need to protect land and aquatic environments that harbor unique species and the potential benefits for economic activities such as ecotourism.

BASELINE FOR CLIMATE CHANGE: MODELING FISH SPECIES DIVERSITY IN WATERSHEDS. Eugene G. Maurakis<sup>1,2,3</sup>, Summer Schultz<sup>1</sup>, and David V. Grimes<sup>1</sup>. <sup>1</sup>Science Museum of Virginia, 2500 W. Broad St., Richmond, VA, 23220, <sup>2</sup>Biology Dept., University of Richmond, and <sup>3</sup>Dept. of Environmental Science and Policy, George Mason University. Objectives are to model fish species richness, diversity and evenness in watersheds of Quantico Creek (a pristine undisturbed

drainage) and Cameron Run (a highly developed urban drainage) using biological (e.g. macroinvertebrate richness and abundance, allochthonous detritus concentration), and physio-chemical factors (e.g. pH, temperature, stream order, width, depth, current, flow, elevation, gradient, river km, substrate composition, land use, and human population per intra-drainage stream order area. To date, 30 species of fishes representing 10 families, including *Channa argus*, the snakeead fish, have been collected from 23 sampling sites over a 6-month period of the two-year study. Funded by U.S. Department of Energy grant DE-FG0208ER64625.

GIS GAP ANALYSIS OF FRESHWATER AQUATIC RESOURCES (FAR) AND FRESHWATER PROTECTED AREAS (FPA) IN GREECE. David V. Grimes<sup>1</sup> and Eugene G. Maurakis.<sup>2,3,4</sup>, <sup>1</sup>Virginia Department of Environmental Quality, 4949 – A Cox Rd., Richmond, VA 23060, <sup>2</sup>Science Museum of Virginia, 2500 W. Broad St., Richmond, VA 24542, <sup>3</sup>Biology Dept., University of Richmond, VA 23173, and <sup>4</sup>George Mason University. Objectives are to quantitatively inspect overlays of FAR and FPA to determine their percent coincidence; Describe the current level of FAR protection in Greece; and Develop practicable recommendations for increasing FAR protection. Spatial analysis of ring buffered FPA, intersected with collection point data for freshwater fishes and the freshwater crab Potamon sp. (FAR), was used to determine the frequency of occurrence of FAR relative to the distance of collection from FPA. Pearson correlation coefficients indicate there is little correlation between the frequency of FAR collection and the distance of the collection point from FPA. Targeted sampling of FPA is needed to determine if FPA in Greece are providing the requisite levels of protection for FAR, particularly fishes listed as extinct, critically endangered, endangered, threatened or vulnerable. We recommend targeted sampling of FPA, urban, rural, resort, agricultural and other land use areas as well as the inclusion of other environmental and anthropogenic variables into a GIS GAP analysis of FAR in order to fully identify their protection needs and environmental quality indicator status.

#### **Materials Science**

(Met with Astronomy, Math & Physics)

Microbiology and Molecular Biology

(Met with Biology)

#### **Medical Sciences**

AUTOLOGOUS PLATELET GEL: A NOVEL TREATMENT FOR MYOCARDIAL INFARCTIONS (HEART ATTACK). C. W. Gurnee<sup>1,2</sup>, B.Y. Hargrave<sup>1,2</sup>, S.J. Beebe.<sup>1</sup> & X. Shu<sup>1</sup>, <sup>1</sup>Frank Reidy Research Center for Bioelectrics, Norfolk VA 23510 and <sup>2</sup>Old Dominion University, Norfolk VA 23529. Autologous Platelet Rich Leukocyte Plasma (PRLP) or "platelet gel" as it is sometimes called is a platelet/leukocyte rich concentrate made from the whole blood of a patient. PRLP, when applied to soft tissue wounds, enhances healing by placing a high concentration of growth factors (released from platelets activated by a known platelet activator) at the site of damage. We examined PRLP in the rabbit heart after Acute Myocardial Infarction (AMI) and reperfusion and tested its ability to support and/or improve mechanical left ventricular function. In Vitro study: In the rabbit Langendorff heart treated with PRLP (injected into the myocardium of the left ventricle) and exposed to global ischemia there was a shift in systolic and diastolic pressure curves suggesting less systolic and diastolic dysfunction compared to the saline treated controls. The PRLP treated but not the saline treated hearts were capable of increasing left ventricular work function to a level above baseline after 40 min of reperfusion. In Vivo study: Fourteen days after an AMI, the rabbit heart treated with PRLP (injected into the myocardium of the left ventricle) at the time of the infarct was stressed with dobutamine and was capable of increasing left ventricular positive dP/dt and decreasing negative dP/dt compared to the saline treated heart. PRLP supports mechanical left ventricular function in the rabbit heart following AMI. These preliminary data suggest that PRLP, injected into the myocardium may function to regulate left ventricular pressures and improve function following AMI.

GENE EXPRESSION OF SACCHAROMYCES CEREVISIAE EXPOSED TO COMMERCIAL WOOD PRESERVATIVES BY DNA MICROARRAY ANALYSIS AND RT-PCR. Madison M. Stevens & Consuelo J. Alvarez, Dept. of Biol. and Environ. Sciences, Longwood Univ., Farmville VA 23909. pentachlorophenol (PtCP) are commercial wood preservatives regulated by the EPA because of their toxicity to wildlife and their possible role as human carcinogens. This baker's yeast was used as a model system to observe changes in gene expression after exposure to these compounds. Cells were exposed to a creosote concentration of 50ng/ml and to a PtCP concentration of 50μM. A total of 20 DNA microarray chips were tested (7 creosote chips, 7 PtCP chips, and 6 solvent chips (used as controls)). 27 genes from creosote and 180 genes from PtCP were found to have significant changes in expression and among them, 15 genes' RNAs were chosen for reverse transcription and RT-PCR to validate their change in expression. In both experimental treatments, genes with roles in cell cycle regulation, drug transport, and response to stress had significant changes in expression. Clustering analysis revealed highly correlated gene expression in genes associated with mitotic controls. Because creosote and PtCP have been indirectly linked to causing cancer in humans, BLASTn and BLASTp analysis on the National Center for Biotechnology Information (NCBI) website was used and confirmed that some genes with significant changes in expression had homology to human genes and protein sequences. Overall, the results of this study are a sign of the necessity for more studies to be done by the EPA and workers' health associations in

order to establish job/health regulations and it could be a starting point for R-1 institutions that concentrate their research in cancer studies.

MBD2 REGULATED CANDIDATE GENES FOR MODULATION OF HUMAN GAMMA GLOBIN GENE EXPRESSION IN ADULT ERYTHROID CELLS. Merlin N. Gnanapragasam<sup>1</sup>, Jeremy W. Rupon, Shou Zhen Wang<sup>4</sup>, Latasha C. Redmond<sup>1</sup>, Omar Y. Mian<sup>3</sup>, Catherine I. Dumur, C.I.<sup>5</sup>, Kelly J. Archer<sup>5</sup>, Joyce A. Lloyd<sup>1</sup> & Gordon D. Ginder<sup>1,2,4</sup> Departments of <sup>1</sup>Human and Molecular Genetics, <sup>2</sup>Internal Medicine, <sup>3</sup>Microbiology and Immunology, <sup>5</sup>Pathology, <sup>5</sup>Biostatistics, <sup>4</sup>Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Reexpression of the silenced fetal  $\gamma$ -globin gene in adult erythrocytes of individuals with  $\beta$ -globin disorders such as sickle cell anemia and β-thalassemia, is of therapeutic interest due to its ameliorating effects<sup>1-5</sup>. We have previously shown that knock out of methyl CpG binding domain protein 2 (MBD2) in transgenic mice carrying the human beta globin gene cluster (β-YAC mice), results in de-repression of γ globin gene expression in adult erythrocytes. However, MBD2 does not directly bind to the γ-globin gene to mediate its silencing. We hypothesize that MBD2 may suppress human  $\gamma$  globin gene transcription in adult erythrocytes by an indirect mechanism ie., by binding to and repressing transcription of intermediary gene/s which may be involved in γ globin gene regulation. Microarray assays were performed on Affymetrix GeneChip® 430A 2.0 array for protein coding genes using RNA from four MBD2-/- and wild type mice adult erythroid cells. Growth factor receptor bound protein 2-associated protein 1 (GAB1) and Zinc finger and BTB domain containing 32 (ZBTB32) were identified as priority candidate genes. Functional studies indicate that overexpression of these validated candidate genes can cause reexpression of y-globin gene in Chemical Inducer of Dimerization dependent β-YAC mouse adult bone marrow cells.

THE PREVENTION OF **EPILEPTOGENESIS** THROUGH **CALCIUM** MODULATION IN A HIPPOCAMPAL NEURONAL CULTURE MODEL OF STATUS EPILEPTICUS-INDUCED ACQUIRED EPILEPSY. Nisha Nagarkatti, Laxmikant S. Deshpande & Robert J. DeLorenzo, Dept. of Neurology, VA Commonwealth Univ, Richmond VA 23298. Currently, no treatment exists to prevent the development of acquired epilepsy (AE) following injury such as status epilepticus (SE). In this study, the ability of a new drug, carisbamate, to prevent the development of spontaneous recurrent epileptiform discharges (SREDs) and alter intra-neuronal Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) following in vitro SE was evaluated. After treatment in low-Mg<sup>2+</sup> containing solution to mimic SE, neuronal cultures developed SREDs (in vitro AE). Cultures were treated, post-SE, with carisbamate (200 µM) for 12 h. The drug was then washed out of the system and neurons were evaluated for the expression of SREDs 24 h post-washout. Drug-treated neurons failed to display SREDs, in contrast to the controls. The ability of carisbamate to block elevations in [Ca<sup>2+</sup>], after SE was also tested because alterations in Ca<sup>2+</sup> dynamics and homeostatic mechanisms have been associated with plasticity changes and ultimately, the development of epilepsy. Following SE, sustained elevations in [Ca<sup>2+</sup>], were observed and carisbamate was able to lower [Ca<sup>2+</sup>], when administered post-SE. When evaluated for the ability to restore Ca2+ homeostasis following glutamate challenge, the drug-treated neurons showed enhanced recovery. This study suggested that carisbamate is able to prevent the development of SREDs in vitro; furthermore, the ability of carisbamate to alter Ca<sup>2+</sup> dynamics may contribute to its anti-epileptogenic properties. Supported by: NIH grants RO1NS051505, RO1NS052529, UO1NS058213 and AHA Pre-doctoral fellowship.

MODELING PHYSICAL ACTIVITY IN WORKING ADULTS: HOW SUITABLE IS THE EXPANDED PARALLEL PROCESS MODEL?. A. B-H-Sam 1, M. L. Walker<sup>2</sup>, S. Plichta<sup>1</sup>, and G. Maihafer<sup>2</sup>; <sup>1</sup>School of Community and Environmental Health, College of Health Sciences, Old Dominion University and <sup>2</sup>School of Physical Therapy, College of Health Sciences, Old Dominion University. The usefulness of the Expanded Parallel Process Model in predicting health enhancing physical activity is assessed in the context of risk for coronary heart disease. The study involves secondary analyses of a dataset from a group of working adults who elected first to participate in a health plan 'Quality Improvement Study' and were then randomly selected to receive an intervention program designed to increase activity. Data on self-reported demographics, physical activity levels, health status characteristics and perceptions measured on a Likert-type scale known as the Risk Behavior Diagnosis Scale are analyzed. The Risk Behavior Diagnosis Scale measures represent the model hypothesized mediating variables which are perceived severity, perceived susceptibility, perceived response efficacy and perceived self-efficacy. Results of data analyses offer limited and weak support for use of the Expanded Parallel Process Model to explain differences in health enhancing physical activity behavior of working adults. The magnitude of the hypothesized Expanded Parallel Process Model mediator variables observed in the study, though small, may suffice as a call for further research using a different research approach (longitudinal). Health behavior is complex, and the most important determining factors of physical activity may also not have been included in the analysis. A different theoretical model, in this case, may help to explain physical activity behavior.

PATTERNS OF ETHANOL-RESPONSIVE GENE EXPRESSION IN FYN KINASE KNOCKOUT MICE. Sean P. Farris & Michael F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. The molecular mechanisms underlying alcoholism are largely unknown, however, changes in gene expression are proposed as critical molecular adaptations leading to the development lasting ethanol related phenotypes. Two inbred mouse strains with divergent ethanol related behaviors, DBA/2J (D2) and C57BL/6J (B6), also exhibit differing basal and acute ethanol-responsive gene expression patterns among discrete brain regions including prefrontal cortex (PFC). Bioinformatic analysis of D2 and B6 microarray studies implicated Fyn kinase as a potential mechanism regulating ethanol-responsive myelin gene expression. Fyn knockout (KO) mice have abnormal CNS myelination and are more sensitive to ethanol related behaviors. Using DNA microarrays we assayed Fyn KO mouse PFC to identify downstream basal and acute ethanol-responsive gene expression patterns. Characterizing the associated gene networks will test the hypothesis that Fyn is required for acute ethanol regulation of myelin gene networks, and identify novel Fyn related signaling mechanisms. Microarray analysis revealed 565

genes altered by genotype, and 746 genes altered by ethanol (P<0.001). Several genes with a functional relationship to myelin including progesterone receptor (Pgr) were regulated by ethanol in Fyn KO PFC, suggesting a novel relationship may exist between Fyn and Pgr in regulating ethanol-responsive myelin gene expression. Continued investigation of this functional relationship and associated gene networks may aid in the future development of more successful pharmacotherapies for alcoholism and related CNS myelin toxicity.

NANOSECOND PULSED ELECTRIC FIELDS INDUCE APOPTOTIC-LIKE CELL DEATH IN MURINE E4 SQUAMOUS CARCINOMA CELLS BY MULTIPLE MECHANISMS. Wei Ren & Stephen J. Beebe, Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk VA 23510. Nanosecond pulsed electric fields (nsPEFs) are pulses with ultra-short duration (ns), high power (mega watts), but low energy density (mJ/cc), which are distinctly different from conventionalelectroporation pulses. NsPEFs have emerged as a novel method to modulate intracellular structures and functions. To determine the signaling pathways in nsPEFinduced cell death, murine E4 squamous carcinoma cells were exposed to multiple pulses from 0 to 60kV/cm with 300ns duration. Cell death occurred with decreases in the mitochondria membrane potential, the appearance of active caspases, and the release of cytochrome c from the mitochondria into the cytoplasm. Using a cell permeable, irreversible inhibitor, the appearance of active caspases was observed within1 hour post pulse. At lower electric fields, active caspases appeared in the apparent absence of cytochrome c release. However, at higher electric fields cytochrome c release was observed. Using irreversible inhibitors of active caspases, active caspase-8, caspase-9 as well as caspase 3/7 were seen within 2 hours post pulse in an electric-field dependent manner. In addition, inhibition of caspase activity using z-VAD-fmk partially attenuated cytochrome c release. These results suggest that nsPEFs induce an apoptotic-like cell death as indicated by using both mitochondriadependent and -independent mechanisms in E4 cells.

METHYL BINDING DOMAIN PROTIEN 2 (MBD2) MAINTAINS TUMOR PROMOTES SUPPRESSOR SILENCING ANDEPITHELIAL DEDIFFERENTIATION IN BREAST CANCER. O.Y. Mian, M. N. Gnanapragasam, S. Z. Wang & G. D. Ginder, Massey Cancer Center, Richmond, VA 23298. Methyl-CpG Binding Proteins (MCBPs) function as interpreters of epigenetic signals encoded in the genome. We study the function of Methyl-Binding Domain Proteins (MBDs) in human mammary epithelial cancers, where repatterning of CpG methylation is common. We find Methyl Binding Domain Protein 2 (MBD2) promotes abnormal multi-cellular morphology of tumor cells grown in extracellular matrix extracts. Stable MBD2 knockdown in MCF7 cells leads to an increased proportion of differentiated epithelial structures (e.g. acinii, 70%, [CI=0.55-0.83]) when compared with untransfected (46%, [CI=0.39-0.53],  $p \le 0.038$ ) and scrambled shRNA transfected (37%, [CI=0.29-0.45],  $p \le 0.012$ ) control cells. To identify the genes underlying this MBD2 dependant phenotype, high throughput quantitative PCR data were probed using self organizing map (SOM) analysis. We found a small subset of the breast cancer specific

tumor suppressors known to be silenced by promoter hypermethylation were regulated by MBD2 (n=7, 15%). The MBD2 dependant genes were rapidly re-suppressed upon rescue with a shRNA binding site variant MBD2 and ChIP studies confirmed binding of MBD2 at genes within the MBD2 dependant cluster. We demonstrate MBD2 maintains aberrant dedifferentiation in breast cancer through a network of epigenetically inactivated tumor suppressor genes. Based on these findings, we intimate a pathologic role for MBD2 in the initiation and progression of human mammary epithelial neoplasia. This work was supported by NIH-2R01DK029902-26A2

NEUTROPHIL INFILTRATION AND RELEASE OF REACTIVE OXYGEN SPECIES ENHANCE VASCULAR REACTIVITY TO ANGIOTENSIN II VIA THE RhoA KINASE PATHWAY. Nikita Mishra, MD, Scott Walsh, PhD, OB/GYN/Physiology, VCU, Richmond, VA 23298. Women with preeclampsia have enhanced vascular reactivity to Angiotensin II (Ang II). We hypothesized that neutrophil release of ROS enhances vessel reactivity to Ang II by activating the RhoA kinase pathway. Resistance arteries from omental fat biopsies of normal pregnant patients undergoing C-sections (n=20) were studied. Ang II dose response (10-9M to 10<sup>-5</sup> M) was done. The Ang II dose response was repeated with neutrophils (2000/mm<sup>3)</sup> in the vessel lumen. Ang II + neutrophils was repeated with addition of superoxide dismutase (150 U/ml)/catalase (5000 U/ml) to quench ROS or with addition of Y-27632 (3μM), a specific Rho A kinase inhibitor. Ang II dose response was also tested with ROS generating solution (hypoxanthine, 0.36mM + xanthine oxidase, 0.004 IU/ml), Ang II + ROS + SOD/catalase or Ang II + ROS + Y-27632 (3µM). Ang II caused a dose response contraction with maximum response at 10<sup>-6</sup>M (Change in diameter of -18.6  $\pm$  2.0m, mean  $\pm$  S.E). With activated neutrophils, vasoconstrictive response to Ang II was significantly greater at 10<sup>-9</sup>M, 10<sup>-7</sup>M, 10<sup>-6</sup>M and 10<sup>-5</sup>M (-44.5 ± 5.9m, p<0.01). SOD/catalase and Y-27632 significantly blocked the enhanced response to Ang II by activated neutrophils. Similar results were observed with ROS. These results suggest that neutrophil infiltration in systemic vasculature of preeclamptic women explain the enhanced reactivity to Ang II in preeclampsia. HL069851.

POSITIVE ALLOSTERIC MODULATION OF  $\alpha4\beta2$  NEURONAL nAChRs BY DESFORMYLFLUSTRA-BROMINE AND ITS ANALOGS. N. A. German¹, J.-S. Kim¹, A. Pandya², M. Schulte² & R. A. Glennon¹,¹Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 and ²Department of Chemistry and Biochemistry, University of Alaska, Fairbanks AK 99775. Neuronal nicotinic acetylcholine receptors (nAChRs) appear to play an important role in cognitive and attentive processes. An alteration in expression or function of  $\alpha4\beta2$  nAChRs also has been associated with neurodegenerative disorders and in certain aspects of drug abuse. Positive allosteric modulators (PAMs) have been envisioned as a potential therapeutic treatment; however there is a very limited number of compounds, if any, that have shown selective allosteric modulation of  $\alpha4\beta2$  nAChRs. A novel indole alkaloid desformylflustrabromine (dFBr), recently isolated from a marine species, has been shown to selectively modulate  $\alpha4\beta2$  nAChRs through an

allosteric mechanism. The present investigation was designed to determine structural features important for the actions of dFBr. Proposed compounds were prepared using several synthetic schemes. Biological activities of synthesized compounds were evaluated using two-electrode voltage clamp techniques employing *Xenopus laevis* oocytes injected with cDNAs of the human  $\alpha 4\beta 2$  receptor. As a result of this study dFBr and its synthetic analogs were shown to be positive allosteric modulators of  $\alpha 4\beta 2$  nAChRs and key structural features were identified for this action. [Supported, in part, by a Virginia Center on Aging grant and a VCU Department of Pharmacology and Toxicology Training Grant (T32 DA007027-34).]

THE EFFECT OF SUCRALOSE ON OBESITY AND DIABETES PROGRESSION IN A TYPE II DIABETES MODEL, THE TALLYHO/JNGJ MOUSE. Matthew C. Johnson & Dianne M. Baker, Dept. of Bio. Sci., Univ. Mary Washington, Fredericksburg, VA 22401. The high incidence of type II diabetes is one of the most pressing medical concerns in the United States. Treatment of type II diabetes commonly includes management of blood glucose and body weight through diet and exercise. To regulate blood glucose, type II diabetics often replace dietary sugars with artificial sweeteners such as sucralose (Splenda®). While sucralose is typically considered to be a safe replacement for sugar, some recent studies have found adverse effects on glucose balance and food consumption. In this study, we tested the effects of sucralose on the progression of type II diabetes in mice. We hypothesized that the presence of sucralose in the diet of type II diabetic mice would accelerate the progression of the disease, resulting in increased glucose levels compared to diabetic control animals. Secondly, we hypothesized that sucralose in the diet would increase food consumption and therefore increase body mass in type II diabetic mice compared to control-fed type II diabetic animals. To test these hypotheses, we measured food intake, body mass, and blood glucose levels (both fasting and glucose tolerance test levels) in sucralose-fed and control-fed animals over a 10 week treatment period. Plasma samples were also collected over this same period for measurement of insulin and triglyceride levels. Contrary to expectations, sucralose-fed mice had lower blood glucose and triglyceride levels than control-fed mice. Additionally, we found no significant effect of sucralose on insulin concentration, food intake or body mass. These results suggest that sucralose may slow the progression of type II diabetes.

THE EFFECTS OF  $\Delta^9$ -TETRAHYDROCANNIBINOL, THE MAJOR PSYCHOACTIVE COMPONENT OF MARIJUANA, ON FOOD AND BRAIN REWARD. M. A. Rolfes<sup>1,2</sup>, A. J. Kwilasz<sup>2</sup>, L. S. Harris<sup>2</sup>, R. E. Vann<sup>1,2</sup>, Departments of Psychology, & Pharmacology/Toxicology, VCU, Richmond, VA, 23298. Previous studies in rats using progressive ratio (PR) schedules of reinforcement have suggested a role for cannabinoid receptor 1 (CB1) agonists in both food consumption and feeding motivation. PR procedures that assess motivation commonly use food reinforcement, however these procedures are unable to delineate whether motivation to respond is an enhancement of feeding or reward mechanisms. Intracranial self-stimulation (ICSS), a procedure in which animals are trained to respond for stimulation of the medial forebrain bundle, is well suited to investigate motivation to respond for reward. Accordingly, the CB1 agonist,  $\Delta^9$ -tetrahydrocannabinol (THC), was assessed for its

ability to alter motivation to respond for food or brain stimulation reward (BSR). ICR mice were trained to respond for food or BSR (158 Hz, 150 µA) on a PR2 schedule of reinforcement, in which the response requirement increased by 2 lever presses after every 4 reinforcers earned. Breakpoints were assessed daily to measure motivation to respond for reinforcement and tests with vehicle and THC (1, 3, 10, and 17.1 mg/kg) were conducted. THC administration significantly increased breakpoints for food and BSR; however, breakpoint increases for BSR were observed at a higher dose than for food. Operant response rates were unaffected by THC. These results add to a growing body of literature that suggests an enhancing role for CB1 agonists in feeding and reward motivated behavior in mice.

THE DOMESTIC FOWL (GALLUS) AS A MODEL OF OBESITY AND SEX SPECIFICITY IN THE METABOLIC SYNDROME. R.P. Wyeth<sup>1</sup>, A. Santo<sup>1</sup>, K.E. Harris<sup>2</sup>, T.V. Palacios<sup>1</sup>, R.M. Lewis<sup>3</sup>, C.F. Honaker<sup>3</sup> & P.B. Siegel<sup>3</sup>, <sup>1</sup>Edward Via, Virginia College of Osteopathic Medicine, <sup>2</sup>Dept. of Human Nutrition, Foods and Exercise, <sup>3</sup>Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg. High body fat content is associated with increased morbidity and mortality. The prevalence of overweight or obese humans grows with alarming rapidity. Globally 1.7 billion people are overweight or obese producing a pandemic of cardiovascular disease (CVD). Principal risks for CVD include abdominal obesity, hyperlipidemia, dyslipidemia, hyperglycemia, insulin resistance, and hypertension, known collectively as the metabolic syndrome. The ability to define mechanisms that interact to produce the metabolic syndrome in humans is limited by lack of a single mammalian model that fulfills all criteria for this syndrome. We propose a suitable alternative. A line of chickens developed at Virginia Tech was selected for high juvenile body weight that not only exhibits rapid juvenile growth but also becomes morbidly obese unless feed restricted. Thus, these high weight chickens provide an attractive model for studying CVD associated with the metabolic syndrome. Based on the metabolic characteristics and preliminary data on these high weight chickens, by inducing obesity, we produced a sexually differentiating effect in the  $\alpha_1$ adrenergic response of this proposed non-mammalian model of the metabolic syndrome. This study was funded by a grant from the Harvey Peters Foundation.

THE FLAVONOID LUTEOLIN INCREASES VASODILATATION THROUGH NON-GENOMIC NITRIC OXIDE RELEASE. Hongwei Si¹, Dongmin Liu¹ & Richard P. Wyeth², ¹Laboratory of Molecular Nutrition, Department of Human Nutrition, Foods and Exercise, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, ²Divisions of Anatomy and Physiology, Edward Via Virginia College of Osteopathic Medicine, Blacksburg, Virginia. Luteolin is a plant flavonoid and vasodilator. We tested if luteolin will stimulate endothelial NO synthase (eNOS) phosphorylation and decrease alpha₁ adrenergic ( $\alpha_1AR$ ) contraction, in cultured human aortic endothelial cells (HAECs) and isolated rat aorta. When intact rat aortic rings were constricted with the specific  $\alpha_1AR$  agonist phenylephrine, followed by cumulative addition of luteolin, a dose dependent relaxation was produced. In rings pretreated with the eNOS inhibitor, N-nitro-L-arginine methyl ester, luteolin–induced vasorelaxation was partially blocked. When HAECs were incubated

with luteolin, luteolin phosphorylated eNOS and stimulated NO release. These data indicate that, within arterial vasculature, luteolin: 1) induces vasorelaxation through an endothelium dependent mechanism; 2) produces a dose dependent and immediate decrease in  $\alpha_1AR$  induced contraction; 3) increases the phosphorylation of eNOS and subsequently improves NO production. Taken together, we suggest that luteolin induces vasorelaxation not by increased eNOS translation but rather by receptor-mediated stimulation of NO production by extant eNOS.

## Natural History & Biodiversity

SMALL MAMMALS FROM THE CLOUD FOREST AT CERRO BOBI, SIERRA DE LOS CUCHUMATANES, GUATEMALA. Nicte Ordonez<sup>1</sup>, Walter Bulmer<sup>2</sup>, Ralph P. Eckerlin<sup>2</sup>, & John O. Matson<sup>3</sup>, <sup>1</sup>Dept. Biol. Sci., Texas Tech Univ., Lubbock, TX 79409, <sup>2</sup>Div. of Nat. Sci., Northern VA Community Coll., Annandale, VA 22003, <sup>3</sup>Dept. Biol. Sci., San Jose State Univ., San Jose, CA 95192. Very little is known about small mammal ecology and distribution in the highlands of Guatemala. Small mammals were removal trapped from the mixed hardwood/coniferous cloud forest at Cerro Bobi in the Sierra de los Cuchumatanes, Huehuetenango, Guatemala during July 2005 and December 2005/January 2006. The coniferous cloud forest is located at 5km SW San Mateo Ixtatan, NW side of Cerro Bobi (3110m). The habitat can be characterized as follows: overstory of fir (Abies), pines (Pinus), oaks (Ouercus), and other broad-leaved trees. A heavy litter of moss, lycopsids, ferns, and fallen logs was on forest floor. A total of 131 individuals representing 10 species of small mammals (8 rodent and 2 shrew) was collected. The site was sampled during two distinct seasons (wet and dry). While there were small differences in the small mammal species composition and abundance between the 2 samples, this was attributed to small sample size and sampling error. Peromyscus guatemalensis was the most abundant species in both seasons. Especially important is the collection of the Maya mouse (Peromyscus mayensis), not formally reported since its original description in 1975.

IDENTIFICATION OF CRYPTIC CHLOROPHYTES THROUGH MOLECULAR SEQUENCE DATA. Matthew R. Semcheski, Todd A. Egerton & Harold G. Marshall, Department of Biological Sciences, Old Dominion University, Norfolk VA 23529. The phenomenon of phenotypic plasticity is evident in many organisms throughout the natural world and is a byproduct of the biotic and abiotic factors of the environment in which an individual or population inhabits. Plasticity is especially prominent among microscopic photosynthetic taxa, which produce a variety of ambiguous forms. We identified a number of morphologically variable specimens, all originating from a single monoculture of the chlorophyte Scenedesmus sp., which included single-cell spinous and non-spinous forms, along with mulitcelluar spinous and non-spinous forms. In order to discern plasticity vs. genetic variation in a number of ecomorphs of Scenedesmus sp., the complete ITS-1, 5.8S, and ITS-2 region was sequenced. Phylogenetic analyses confirmed that all samples analyzed, morphologically distinct, do indeed belong to the Scenedesmus genus. Upon further investigation, it was determined that at the outset, with nutrients non-limiting and an absence of predators, Scenedesmus sp. grew rapidly in the single-cell non-spinous form.

Over time, a population of rotifers had contaminated the Scenedesmus sp. cultures, triggering the production of multicelluar, spinous forms, a typical anti-predator strategy for this genus. It is then concluded that Scenedesmus sp. does exhibit phenotypic plasticity in response to biotic and abiotic changes in the surrounding environment. Additionally, some genetic variation was found in several other isolates from a pure Scenedesmus sp. culture, all of which, group within the Scenedesmus-Desmodesmus complex.

SALAMANDER DIVERSITY AT C. F. PHELPS WILDLIFE MANAGEMENT AREA, FAUQUIER AND CULPEPER COUNTIES, VIRGINIA. J. D. McGhee & M. D. Killian, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22407. Salamander guilds are important components of ecosystems, and may be declining in Virginia. Consequently baseline information on salamander diversity and abundance is needed. Our objective was to assess salamander diversity at a single site in the Rappahannock River watershed, C. F. Phelps Wildlife Management Area. We randomly selected stream and upland terrestrial sites to run 50meter transects, for both quadrat and natural cover searches. We assessed diversity using a Shannon-Weiner index on all captures and non-larval captures, compared findings quantitatively to similar studies, and assessed diversity in on-site watersheds. We found 11 of 13 expected species, with  $H' = 1.33 \pm 0.05$  SD, J' = 0.55, and for nonlarval diversity  $H' = 1.18 \pm 0.08$  SD, J = 0.49. The slope of captures per species was similar to other studies,  $\beta_1 = -0.50 \pm 0.10$ . A single watershed (Fishing Run stream) was considered more diverse than other watersheds on site. We conclude that C. F. Phelps Wildlife Management Area supports a relatively diverse salamander community, and may act as a baseline for the surrounding region. Management efforts should be focused to maintain stream structural diversity, and monitoring agricultural input.

GEOGRAPHIC INFORMATION SYSTEMS AUGMENT MONITORING IN DAM REMOVAL PROJECT. Alan B. Griffith & Damon Lowery. Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, Virginia, 22401. As dam removals have increased in frequency, due to dam deterioration and interest in ecosystem restoration, there is a growing need to determine the ecological effects of dam removal. Few studies have been conducted on dam removals and pre-dam removal data is particularly limited. We present here a case study that explores the use of Geographical Information Systems (GIS) to supplement vegetation sampling data on the ground. Our part of an interdisciplinary study aims to measure changes in distribution and abundance of vegetation after the removal of 2 earthen dams on a tributary of Holts Creek in New Kent County, VA. We previously reported results of plant distributions and abundances measured before the removal of these dams. Our sampling revealed that at least one invasive species, Murdannia keisak and Microstegium vimineum were broadly distributed and in high abundance within the dams' watershed. As dam removal proceeds, it will be essential to monitor the establishment of these invasive species and other species. Based on our current knowledge of GIS applications we 1) will visualize vegetation samples as spatially related information, 2) have gathered existing geo-referenced information for the

impacted watershed, and 3) have uncovered potentially useful spatially related information, for which no data exists. This process has led us to collect geo-referenced data that will improve our ability to monitor vegetation changes after dam deconstruction.

PHYTOPLANKTON DIVERSITY TRENDS IN THE RAPPAHANNOCK, YORK AND JAMES RIVERS. Todd A. Egerton & Harold G. Marshall. Dept. of Biological Sciences, Old Dominion University. Norfolk VA 23529. The examination of the causes and consequences of biodiversity is a central tenet in ecological research. In estuarine habitats, one factor which has been shown to have an effect on diversity is salinity. Remane (1934) described the change in community composition of benthic invertebrates along a salinity gradient and the accompanying change in species richness. He observed the greatest numbers of species in the freshwater and marine portions, and the lowest in the brackish mesohaline region. This study examines the diversity of phytoplankton species along the York/Pamunkey, and James rivers, tidal tributaries to Chesapeake Bay. These three rivers have varying levels of algal productivity and diversity, and show the same general pattern as described by Remane, with reduced species richness in the mesohaline. Additionally, there is a significant positive correlation between phytoplankton diversity and productivity, but only in the upstream, low salinity stations. This may be attributed to the large seasonal fluctuations in phytoplankton abundance in the freshwater sites, and the relative constancy of the populations in the Chesapeake Bay. This study is based on the long-term phytoplankton monitoring data, gathered as a component of the Chesapeake Bay Program.

PHYLOGENY OF THE CARYOPHYLLALES (ANGIOSPERMS): EXPLORING THE EFFECTS OF GENE CHOICE AND MISSING DATA. Sunny S. Crawley, Shelli A Newman & Khidir W Hilu, Department of Biological Sciences, Virginia Tech, Blacksburg VA 24061. Previous work on reconstructing Caryophyllales phylogeny has relied on data from two to many genomic regions, totaling 3,000 to 46,000 base pairs of sequence information. Topology, resolution, and support for the internal structure of the order have varied, but improvement has been noted as the number of genes/characters increased. We explore here the impact of gene choice and degree of missing data on tree topology and support within Caryophyllales. We chose two rapidly evolving regions (matK and surrounding trnK intron), two slowly evolving regions (atpB and rbcL) and one with an intermediate rate of evolution (ndhF). We supplemented new matK/trnK sequences with complete and partial sequences from GenBank for all genomic regions. Maximum parsimony and maximum likelihood methods were used to analyze 130 species with six basal eudicot species as outgroup. Varying degrees of missing data were analyzed as several different data partitions. The phylogenetic structure of the order recovered with rapidly evolving regions was comparable to that obtained with the three other regions. Topology and support based on combined analysis of five regions was remarkably similar to those obtained using much larger numbers of genes/characters; this was achieved despite having about 46% missing data. Combining genes of different mode of evolution and inclusion of partial

sequences resulted in both increased taxon representation and improved overall phylogenetic structure.

SPATIAL DISTRIBUTIONS OF FISHES AT SPECIES RICHNESS AND NEARSHORE HABITATS, ST. JOHN VIRGIN ISLANDS. Maurakis<sup>1,2,3</sup>. Science Museum of Virginia, 2500 W. Broad St., Richmond, VA 24542, University of Richmond, VA 23173, George Mason University, Fairfax, VA. Objectives were to identify species diversity and delineate distributions of species at sand- and boulder-shoreline beach habitats, and compare similarity of species and feeding type associations to establish a baseline at these two beach habitats. Fish species richness and spacial distributions, and habitat descriptions were surveyed by visual census using snorkel and mask at 1-m intervals from 1-40 m from shore at 20 transects at Little Lameshur, Great Lameshur, and Francis Beach, St. John, U.S. Virgin Islands during daylight hours. A total of 69 taxa (67 species) representing 33 families of fishes were observed. Average number of species (32.7) at boulder beach habitats were significantly greater than that (24.3) at sand beach habitats. The most speciose functional feeding groups were mobile benthic invertivores (11 species at 9 m from boulder shore habitats), scrapers and piscivores (each 9 species at 6 m from boulder shore habitats), and macrocarnivores (6 species at 6 m from sand shore habitats). Total numbers of functional feeding groups (range=10-12) and species per functional feeding groups (range=29-46) at all distances from shore at boulder transects were consistently higher than those (functional group range=8-10; species=19-30) at sand transects.

A SURVEY OF STRUCTURES USED BY RAFINESQUE'S BIG-EARED BATS IN VIRGINIA (Corynorhinus rafinesquii). L.T. Pletcher, S. Murdock & J.D. Kleopfer, Virginia Department of Game and Inland Fisheries. The Rafinesque's Big-eared Bat, a Virginia state-endangered species, is categorized as a species of greatest conservation concern (Tier I). The objective of this study was to determine the distribution and abundance of this species by surveying man-made structures. This 2008 study continues previous inventory efforts begun by the VDGIF in 1993 which have identified individuals and colonies roosting in man-made structures, many of which had not been monitored since 2001. This study was conducted by revisiting previously documented structures and counting the number of individuals present, road cruising for potential new structures and using county GIS information to obtain landowner cooperation. Of the 94 previously documented structures, 23 were confirmed to be in good status and 15 of these had bats present. The fate of 21 structures is unknown, 14 structures have been destroyed since 2002, and 29 were known to be destroyed prior to 2001. There were 4 active maternity colonies, each containing 30 - 50 females and young. Eleven solitary roosts were documented. Approximately 200 individuals were observed, mostly in Southhampton and Sussex Counties and the City of Virginia Beach. No individuals were found in Charles City, Surry, and Prince George Counties, or the City of Petersburg. The overall population status in Virginia is unknown. Continued publicity and education are needed to enlist landowner cooperation and locate other bat roosts.

MITOCHONDRIAL DNA VARIATION IN THE EASTERN FOX SQUIRREL (SCIURUS NIGER). N. D. Moncrief, VA Museum of Natural History, 21 Starling Ave., Martinsville Virginia 24112 & R. A. Van Den Bussche, Department of Zoology, Oklahoma State University, Stillwater OK 74078. The eastern fox squirrel (Sciurus niger) occurs naturally over most of eastern North America. This species displays striking patterns of geographic variation in size and coat color. These patterns of morphologic variation are consistent with a hypothesis of southward range contraction and isolation in two refugia (in Texas and Florida) during the Last Glacial Maximum, followed by northward range expansion after the glaciers receded. Similar hypotheses have been proposed to explain the patterns in phylogeographic structure exhibited by many plants and animals in eastern North America. As part of a more comprehensive study of geographic variation in Sciurus niger, we analyzed a 402 bp segment of the cytochrome b (cyt b) mtDNA gene in populations throughout the species' range. Despite the broad geographic sampling in our study, there was no phylogeographic structure in our data. Unique haplotypes differed from high-frequency haplotypes by only one or two base pairs, producing a star-like phylogeny of haplotypes. Bootstrap analysis of neighbor-joining trees revealed a lack of phylogenetic structure among haplotypes. Variation within populations and within the species as a whole was characterized by high haplotype diversity and low nucleotide diversity. Taken together, our data indicate that the eastern fox squirrel underwent a rapid range expansion and rapid morphological divergence within the past 20,000 years.

## **Psychology**

INTERHEMISPHERIC COLLABORATION: EFFECTS OF STIMULUS FORMAT AND TASK PROCESSING SIMILARITY. Urvi J. Patel, Dept. of Psych., Christopher Newport Univ., Newport News VA 23606. Observers were presented with a five stimuli array; two items above the point of fixation (one to each visual field), one item below the point of fixation (to one visual field), and two items directly above the other at the point of fixation (to both visual fields). During each experiment, observers engaged in three conditions: (1) responded to whether the bottom stimulus matched either of the top two stimuli (single primary task), (2) responded to whether the two center stimuli matched (single secondary task), and (3) responded to the primary stimuli OR to the secondary stimuli as prompted by a post-stimulus cue (dual task). While all letter stimuli were presented for Experiment 1, Experiment 2 displayed letter primary stimuli and picture secondary stimuli. Performance on the single and dual primary trials was of principal interest. The critical comparison involved trials on which the two matching stimuli projected to the same visual field (within-hemisphere trials) versus trials on which the two matching stimuli projected to opposite visual fields (acrosshemisphere trials). While no difference between trials was found when the dual task involved stimuli of different format, an across hemisphere advantage was found when the dual task involved stimuli of the same format. Processing similarity of stimulus format may determine whether the benefits to spreading the processing load between the two hemispheres outweigh the costs.

ARE DIFFERENT PEOPLE SUSCEPTIBLE TO DIFFERENT DISTRACTIONS? THE RELATIONSHIP BETWEEN LEARNING STYLES AND PERFORMANCE UNDER DISTRACTING TASK CONDITIONS. Rachel R. Phillips & Poornima Madhayan, Dept. of Psych, Old Dominion University, Norfolk VA 23529. In order to examine the relationship between learning styles and performance for a complex visual search task under distracted and undistracted conditions, eighty participants completed two complex visual search tasks, one with distraction and one without (the conditions were counter-balanced). Distractions were presented either auditorially or visually, and were either verbal or spatial in nature. This was a 2 (perceptual modality: auditory vs. visual) x 2 (processing code: verbal vs. spatial) x 2 (distraction: distracted vs. undistracted) mixed design. Results for hit rates revealed that participants scoring high in the verbal dimension performed differently than those scoring high in the visual dimension depending on distraction and condition. Hit rates for those classified as sensors varied differently when distracted versus undistracted than those classified as intuitors which also varied as a result of gender. Results for false alarm rates revealed that those in the sensing dimension performed better when distracted but those in the intuiting dimension performed worse. Analyses for false alarms also revealed that males and females performed differently when distracted versus undistracted and that this varied by condition. Finally, in addition to the overall sensing and intuiting dimension differences for false alarms, this also varied with gender and distracted condition. These findings have implications for the development of training programs, computer automation, and contribute to the overall understanding of the influence of individual processing preferences on performance.

EMERGENT LEADERSHIP AND TEAM PERFORMANCE AS A FUNCTION OF TASK DIFFICULTY IN A DISTRIBUTED COMPUTER GAME-BASED ARCHITECTURE. Alexandra B. Proaps & James P. Bliss, Dept. of Psychology, Old Dominion University, Norfolk, VA, 23529. Specialized military field training can be expensive, time-consuming and dangerous. The use of computer game-based architectures may help provide a safe, controlled environment in which geographically dispersed military units (i.e., distributed) can develop decision-making and leadership skills while rehearsing a specific task, such as building clearing, area reconnaissance, or navigation. Leaders of distributed teams need to know how to perform to overcome the challenges involved in these virtual environment contexts. Current research shows there are implications of task difficulty on how distributed team members emerge as leaders within virtual environments. The purpose of the proposed study was to investigate the possible relationship between task difficulty with team task performance and emergent leadership during a team search task using a modified version of the popular video game, Half Life 2<sup>TM</sup>. The experimenters determined that task difficulty decreased the speed with which the task was completed and that the gender composition of the dyad had an effect on accuracy and speed. The experimenters also found dyad members rated each other as sharing the overall contribution to the task. Dyad members did rate one member of the team as the overall leader, but their rating of leadership did not change based on task difficulty.

TRAINING INCENTIVES IN AN INVESTIGATION OF PERFORMANCE ON COMPLEX TASKS. Patricia C. Brennan & Poornima Madhavan, Dept. of Psyc., Old Dominion Univ., Norfolk VA 23507. Incentives not only serve as extrinsic motivation for a particular task, but also manipulate people to behave a certain way. We are interested in studying how framed incentive structures may have training implications in a visual search task such as airline luggage screening. The framed incentive structures are a representation of choices in either a positive (rewards) or negative frame (punishment) in which points are given or taken away to influence detection behavior in finding weapons across two phases - training (familiar targets) and transfer (novel targets). We are only interested in performance during transfer due to the real world implications. Participants are presented with the context of being an airport luggage screener with points for hits, misses, false alarms and correct rejections, which serve as incentive structures. The goal is to improve the design of training programs for operators in applied visual search tasks, particularly airport luggage screening. We found that providing incentives does enhance performance in maximizing hits, in which the hit-sensitive and miss-sensitive outperformed the equal-costs and no-incentives groups. However, the control group outperformed all other groups in transfer because they were able to self-train thus conjure up their own representation of a weapon. In all the control group had the highest rate of hits, highest overall confidence, lowest rate of false alarms and fastest response times.

EXAMINING THE HINDSIGHT BIAS EFFECT DURING JUDGMENTS OF TRUST. Martin D. Smith-Rodden & Ivan K. Ash, Dept. of Psychology, Old Dominion University, Norfolk, VA. 23529. The Hindsight Bias Effect (HSE) describes people's tendency to overestimate their own ability to predict an event once an event's outcome is known. HSE has been shown to be a ubiquitous, reliable, and potentially harmful retrospective judgment bias. The purpose of this research was to examine participants' evaluations of trust outcomes, to determine how judgments of trust might be susceptible to hindsight biases, in two experiments. Subjects were exposed to fictional vignettes about interpersonal trust: one about a small money loan (N=120), and in the second experiment, one depicting a small business hiring decision (N=122). To manipulate subject's surprise at outcome, stories were controlled for congruency (i.e. degree of trustworthiness of target, versus the outcome, in which the target either upholds trust or defects). Subjects were polled for their opinions on the target's trustworthiness just prior to learning the outcome, and again for their recollections of that opinion in a posttest administered exactly one week later. Results were analyzed in a 2 (pretest/post-test) X 2 (Target is trustworthy/Target is non-trustworthy) X2 (Trust upheld/ Defection) mixed design. Increased hindsight bias was observed on unexpected outcomes and no/reverse hindsight bias on expected outcomes in a significant interaction predicted by the Sense-making model of hindsight bias. However, in Experiment 1 we did not observe hindsight bias in the condition where the story's target unexpectedly failed to uphold trust. This may be because participants interpreted the failure to pay back a loan as a "non-event," as previous research has shown abated hindsight bias effects for these types of "non-event" outcomes.

ALCOHOL AND INJURY: DEFINING THE ALCOHOL PROBLEM ON COLLEGE CAMPUSES. Diane A. Kokorelis & Bryan E. Porter, Dept. of Psychology, Old Dominion University, Norfolk VA 23529. Unintentional injuries, particularly motor vehicle crashes, are the leading cause of death among 1-44 year olds. A particularly large portion of these crashes are caused by young males under the influence of alcohol. This study looks to gather and examine the knowledge and attitudes concerning alcohol and drinking and driving among students at a southeastern Virginia university in order to create an appropriate intervention on campus. Data were collected from 310 psychology undergraduates, 199 being female and approximately 55.2% being Caucasian. Multiple correlations and one regression were conducted to assess the relationship between alcohol consumption and the threat and efficacy components of the Risk Behavior Diagnosis Scale concerning the consumption of alcohol as well as drinking and driving. Results indicate that while students' levels of drinking correlate with levels of threat, the same does not hold true for their levels of efficacy. Further, CDC-classified binge drinkers feel as though their behavior does not pose a serious threat, nor do they think they can alter their drinking behaviors. The results also suggest that the more students drink, the more likely they are to drink and drive. Finally, there was no significance found between each gender's uses of a designated driver.

A THEORETICAL MODEL FOR RELIGION'S ROLE ON DOMINANCE DIFFERENCES IN GENDER. Julia R. Quigley, Dept. of Psychology, Christopher Newport University, Newport News, VA 23606. This study presents a theoretical model to explain the potential relationship between religion, gender schemas, and dominance. Social learning theories and research show that religion creates gender schemas with associated dominance differences in the sexes. The prescribed dominance patterns in the sexes could lead to unintended consequences, such as pay scale differences, anorexia, and poor academic performance.

STUCK IN THE MIDDLE WITH YOU: THE EFFECT OF BIRTH ORDER ON CREATIVITY. <u>Laura Boettcher</u> & Gayle Dow, Psychology. Department, Christopher Newport University, Newport News, VA 23606. Birth order has been found to play an important role in how participants perform on creativity measures. The purpose of this study was to investigate the impact of birth order (youngest, middle, and oldest) on verbal measures of creativity. Forty-one undergraduates from a liberal arts university completed a creativity assessment, specifically the Remote Associates Test (RAT), and a measure of demographics, including birth order. There was a significant main effect of birth order on creativity (middle-born out performed youngest-born).

CREATIVITY AND COGNITIVE FLEXIBILITY. <u>Julie F. Erath</u> & Urvi J. Patel, Dept. of Psych., Christopher Newport Univ., Newport News VA 23606. A number of studies have reported a positive relationship between creativity and cognitive flexibility. Creativity may be defined as the ability to use diverse manners of thought to generate novel and dynamic ideas and solutions. Consistent with such a mode of processing, cognitive flexibility is the ability to restructure knowledge in a manner that allows ease in task shifting. The present study was designed to investigate how this relationship may be influenced by verbal ability (i.e., vocabulary knowledge and the

ability to reason by way of such knowledge). Four verbal measures were administered to 47 undergraduate students to evaluate how the following constructs interrelate: creativity (Remote Associates Test), cognitive flexibility (self-report Cognitive Flexibility Scale), and verbal ability (Shipley Vocabulary Test and Homographs Task). Correlation analyses revealed a significant positive relationship between select measures of interest. Specifically, verbal knowledge and reasoning may influence one's ability to generate novel solutions which, in turn, may be associated with adaptability to different tasks. These results are consistent with the findings of previous studies and make a unique contribution to the literature by suggesting that verbal ability may facilitate the relationship between cognitive flexibility and creativity.

AN INVESTIGATION OF EATING PATTERNS AND WOMEN'S BODY OBJECTIFICATION. Whitney N. Kailos, Dept. of Psychology, Christopher Newport University, Newport News, VA 23606. The current study investigates the relation between college women's eating patterns and behaviors in retrospect to certain influential factors such as body objectification. Through the social comparison theory women may either objectify or subjectify their bodies leading to abnormal eating behaviors. College women presumably concern themselves with weight, body scrutiny, and social status which may encourage food restriction and eating disorders. This study used published literature to further examine women in a college setting to determine the reasons behind their eating behaviors.

ASSOCIATION STUDY BETWEEN GABA RECEPTOR GENES AND ANXIETY DISORDERS. Xuan T. Pham & John M. Hettema, Dept. of Psychiatry. Virginia Commonwealth Univ., Richmond VA 23220. Human anxiety disorders are complex diseases with relatively unknown etiology. Dysfunction of the GABA system has been implicated in many neuropsychiatric disorders, including anxiety and depression. In this investigation, we explored four GABA receptor genes for their possible associations with genetic risk for anxiety disorders. Using multivariate structural equation modeling, we selected twin subjects scoring at the extremes of a latent genetic risk factor shared by neuroticism, several anxiety disorders, and major depression from a large population-based twin sample. Our study sample consisted of 589 cases and 539 controls (n=1128), which we subjected to a two-stage association study. In stage 1, all genetic markers were screened, the positive results of which were tested for replication in stage 2. We genotyped altogether 26 single nucleotide polymorphisms (SNPs) from the four GABA receptor genes (GABRA2, GABRG2, GABRA6, or GABRA3). Of the 26 SNPs genotyped in stage 1, we identified 2 markers in the GABRA3 gene that met the threshold ( $p \le .1$ ) to be tested in stage 2. These two markers, along with an additional two, failed to replicate in stage 2. Haplotype associations for this gene showed no significance across all haplotype combinations. Our findings did not show sex-specific associations for any of the markers on GABRA3. Our 2-stage association design did not reveal association for in anxiety disorders. The full extent to which polymorphisms in the GABA system may affect the genetic predisposition for anxiety disorders still remains to be elucidated.

TRAIN YOUR BRAIN TO BEHAVE: CLINICAL APPLICATIONS OF NEUROFEEDBACK. Kathryn. N. Hoey, Dept. of Psychology, Christopher Newport University, Newport News, VA 23606. Neurofeedback (NF) is an operant-conditioning system, known as neuroregulation, which teaches individuals how to control or change their own brain activity. When an individual is diagnosed with a disorder that can be treated via neurofeedback, that patient can then seek out a clinical psychologist or other practicing therapist for neurofeedback treatment. The therapist will begin the treatment with a comprehensive qualitative EEG (qEEG) to gather the data necessary in order to devise a treatment program for that particular patient. Disorders that can be treated using neurofeedback include but are not limited to ADHD, cerebral palsy, migraines, and epilepsy. Each disorder is treated with specific neurofeedback protocols that target specific areas of the brain in order to achieve a specific change in brain functioning.

#### **Statistics**

SUPPORT VECTOR MACHINES WITH THE RAMP LOSS AND THE HARD MARGIN LOSS. J.P. Brooks, Dept. of Stat. Sci. and O.R., Virginia Commonwealth University, Richmond, VA 23284. The support vector machine (SVM) is a well-established method for classification based on an approach that emphasizes minimizing misclassification error while maximizing the distance between sets of correctly classified observations. In training models, SVM uses a measure of error that is based on the Euclidean distance of observations from the separating surface. In the interest of increasing the robustness of SVM, we present two new integer programming formulations that incorporate the ramp loss and the hard margin loss, respectively. These formulations are able to accommodate nonlinear kernel functions that have made traditional SVM popular. The consistency of SVM with these loss functions is established. Analysis of simulated and real-world data sets indicates that Ramp Loss SVM is preferred over both Hard Margin Loss SVM and the traditional Hinge Loss SVM in the presence of outliers when a low-rank kernel function is employed.

EVALUATING STATISTICAL SIGNIFICANCE IN **SUPERSATURATED** DESIGNS. David J. Edwards, Dept. of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, VA 23284 & Robert W. Mee, Dept. of Statistics, Operations, and Management Science, Univ. of Tennessee, Knoxville, TN 37996. Two-level supersaturated designs (SSDs) are designs that examine more than n-1 factors in n runs. Although literature involving the construction of SSDs is plentiful, less has been written about analysis of data from these designs. Perhaps this is due in large part to the dearth of actual applications. Whether using forward selection or all-subsets regression, it is easy to select models from SSDs that explain a very large percentage of the total variation. Hence, naïve p-values can persuade the user that included factors are indeed active. We propose the use of a global model randomization test in conjunction with all-subsets to more appropriately select candidate models of interest. For settings where the number of factors is too large for repeated use of all-subsets to be applied repeatedly, we propose a short-cut approximation for the p-values based on the beta distribution. Finally, we propose a randomization test for reducing the number of terms in candidate models with small global p-values.

USING SIMULATION OPTIMIZATION TO CONSTRUCT **EFFICIENT** SCREENING STRATEGIES FOR CERVICAL CANCER. Laura A. McLay & Chris Foufoulides, Dept. of Stats. & Oper. Res., Virginia Commonwealth Univ. Cervical cancer is the second most common type of cancer in women worldwide. Because cervical cancer is usually asymptomatic until the disease is in its advanced stages, cervical screening is of central importance towards combating cervical cancer. Alternative screening strategies are evaluated from an economic point of view through cost-effectiveness analysis. In the literature, however, studies perform costeffectiveness analysis on a limited number of de facto screening policies. At present, no attempt has been made to construct efficient screening strategies through optimization, before cost-effectiveness analysis is applied. In this study simulation optimization is used to construct efficient screening strategies for cervical cancer by properly timing the screenings. The constructed strategies are highly cost-effective when a small number of lifetime screenings is available, and are more cost-effective than screening strategies used in practice or considered in the literature so far, indicating the value of optimal timing for other screened diseases as well.

EVALUATING THE ASYMPTOTIC LIMITS OF THE DELETE-A-GROUP JACKKNIFE FOR MODEL ANALYSES. Phillip S. Kott, National Agricultural Statistics Service, Department of Agriculture, Fairfax VA 22030 & Steven T. Garren, Department of Mathematics and Statistics, James Madison University, Harrisonburg VA 22807. The delete-a-group jackknife can be effectively used when estimating the variances of statistics based on a large sample. The theory supporting its use is asymptotic, however. Consequently, analysts have questioned its effectiveness when estimating parameters for a small domain computed using only a fraction of the large sample at hand. We investigate this issue empirically by focusing on heavily poststratified estimators for a population mean and a simple regression coefficient, where the poststratification take place at the full-sample level. Samples are chosen using differentially-weighted Poisson sampling. The bias and stability of delete-agroup jackknife employing either 15 of 30 replicates are evaluated and compared with the behavior of linearization variance estimators.

INFORMATION REDUCTION FOR BIAS AND VARIANCE ESTIMATION. Leonard A. Stefanski, Dept. of Stat., N.C. State Univ., Raleigh, NC 27696-8203. The jackknife and bootstrap are two well-known methods of reducing bias and estimating variance. Simulation-extrapolation is a method of reducing bias and estimating variance in measurement error models that works by adding more error to the observed data. Omitting an observation (jackknife), sampling from the observed data (bootstrap), and adding noise to data (simulation-extrapolation) are all ways of reducing information in a data set. In this talk I show that all three methods are conceptually similar when viewed in terms of information reduction, and argue that doing so is sometimes advantageous.

#### BEST STUDENT PAPER AWARDS

#### AGRICULTURE, FORESTRY, AND AQUACULTURE

Brandon Newmyer

The Anorectic Effect of Neuropeptide AF is Associated With Satiety-Related Hypothalamic Nuclei.

Radford University

Honorable Mention

Michele Mohrmann

Nodulation Traits of Tepary Bean Inoculated with 15 Bradyrhizobial Strains Virginia State University

## ASTRONOMY, MATHEMATICS AND PHYSICS WITH MATERIALS SCIENCE

Craig Hanley

Infrared emission properties of Nd: KPb5Br5 for solid-state lasers

Dept of Physics

Hampton University

Honorable Mention

Olusola Oyebobla

Concentration dependent studies of the laser-induced infrared emission from KCl-

NaCl tablets

Dept of Physics

Hampton University

Honorable Mention

Robert Brik

Quantitative Analysis of Background Radiation Particle Tracks in a Large Diffusion

Cloud Chamber Using "ImageJ" Digital Imaging Techniques.

MIT and Science Museum of Virginia

#### BIOLOGY WITH MICROBIOLOGY

GRADUATE

Meghan Durham-Colleran

Initial Report of in vitro Biofilm Formation in Francisella: A Role for an Orphan

Response Regulator

George Mason University

## 110 VIRGINIA JOURNAL OF SCIENCE

#### UNDERGRADUATE

V. O. Chau

Delayed Treatment with Sildenafil Attenuates Ischemic Cardiomyopathy: Role of RhoA/Rho Kinase Pathway

Virginia Commonwealth University

#### BIOLOGY WITH MICROBIOLOGY

Graduate Honorable Mention
Nathan A. Bowman hm
Seasonal Patterns of Phytoplankton Populations in Back Bay, Virginia
Department of Biological Sciences, Old Dominion University

Undergraduate Honorable Mention

Madelyn G. Crowell

Natural Genetic Variation in Metabolic Rate and Activity in White-footed Mice (Peromyscus leucopus) in Relation to Genetic Variation in Reproductive

Photoresponsiveness

Virginia State University

#### BIOMEDICAL AND GENERAL ENGINEERING

E. Heade Spratley

Computational Modeling of Varus Elbow Instability in Terrible Triad Injuries Department of Biomedical Engineering VCU

#### BOTANY

Jessica Weaver (co-author: K. G. Jones)

Isolation and Characterization of Leaf Endophytes in Betula uber and Betula lenta. University of Virginia's College at Wise

#### BOTANY

Harvill Award (\$150.00)
Paige E. Miller (student of John Hayden)
University Of Richmond

#### **CHEMISTRY**

S.W. Chocklett Hydroxamate Formation in Siderophore Biosynthesis Virginia Tech Honorable Mention
Tim Fuhrer
Relative Stabilities Of C94 And C942- Ions Using Quantum And Statistical
Mechanical Functions
Virginia Tech

Honorable Mention

A. E. Rue

Modification of Macroporous Silica Thin Films Via Metal Nanoparticle Deposition Virginia Commonwealth University

#### MEDICAL SCIENCES

Nisha Nagarkatti

The Prevention of Epileptogenesis through Calcium Modulation in a Hippocampal Neuronal Culture Model of Status Epilepticus-induced Acquired Epilepsy Virginia Commonwealth University

Merlin Gnanapragasam
Honorable Mention
The Role of Methyl-CpG Binding Domain Protein2 (MBD2) in the Human Fetal
Gamma Globin Gene Regulation
VCU

Carrie Gurnee
Honorable Mention

Platelet Gel: A Novel Treatment for Myocardial Infarction (Heart Attack). Old Dominion University

Omar Y. Mian Honorable Mention The Methyl-Binding Domain Protein 2 (MBD2) maintains epithelial dedifferentiation in breast cancer VCU, Goodwin Research Lab.

#### NATURAL HISTORY AND BIODIVERSITY

**Todd Egerton** 

Phytoplankton diversity trends in the Rappahannock, York, and James Rivers Department of Biological Sciences Old Dominion University

## 112 VIRGINIA JOURNAL OF SCIENCE

#### **PSYCHOLOGY**

Diane Kokorelis

Alcohol and injury: Defining the alcohol problem on college campuses

Department of Psychology, Old Dominion University

#### **STATISTICS**

S. H. Sathish Indika Latent Model Parameter Estimation and Characterization in a Bivariate Lifetime Distribution Old Dominion University

## VIRGINIA JUNIOR ACADEMY OF SCIENCE MAY 28, 2009 68th ANNUAL MEETING AWARDS

# 68th ANNUAL MEETING AWARDS VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA

#### AGRICULTURE AND ANIMAL SCIENCE

Honorable Mention: KATHERINE CHEN

Mills E. Godwin High School

Honorable Mention: VICTORIA S. FUBARA

Deep Run High School

Honorable Mention: VAISHNAVI KOSURI AND DIVYA MADHUSUDHAN

Thomas Jefferson High School for Science and Technology

Third Place: JORDAN B. HURST

Chesapeake Bay Governor's School

Second Place: MATTHEW T. KING

George H. Moody Middle School

First Place: TIAN ZHOU

Blacksburg High School

### ANIMAL BEHAVIOR (ETHOLOGY)

Honorable Mention: RYAN P. CARROLL

Yorktown High School

Honorable Mention: SEANA HEDAYATNIA

Mills E. Godwin High School

Honorable Mention: ERICA L. STANLEY

Central Virginia Governor's School

Third Place: ADITHYA SIMHA AND KEVIN H. SHU

Thomas Jefferson High School for Science and Technology

Second Place: CARINE L. SQUIBB

Southwest Virginia Governor's School

First Place: JEEIN SEO

Thomas Jefferson High School for Science and Technology

#### **BOTANY A**

Honorable Mention: SARAH N. BOEGNER

George H. Moody Middle School

Honorable Mention: JAKE H. HILL

Central Virginia Governor's School

Honorable Mention: CHRISTOPHER M. NOWAK

George H. Moody Middle School

Third Place: ANNA M. BROSNAHAN

Washington-Lee High School

## 114 VIRGINIA JOURNAL OF SCIENCE

Second Place: SUCHANA H. COSTA

Washington-Lee High School

First Place: MADHURA V. CHITNAVIS

Roanoke Valley Governor's School

**CHEMISTRY A** 

Honorable Mention: SOFONIAS GETACHEW

Williamsburg Middle School

Honorable Mention: ANDREA E. GREEN

H. B. Woodlawn

Honorable Mention: JIMIN HE

Yorktown High School

Third Place: SAUMIL BANDYOPADHYAY

George H. Moody Middle School

Second Place: VIJAY GOVINDARAJAN

Mills E. Godwin High School

First Place: PRASANNA G. JOSHI

Mills E. Godwin High School

**CHEMISTRY B** 

Honorable Mention: FARIS G. SANJAKDAR

Washington-Lee High School

Honorable Mention: PRIYA SARKAR

George H. Moody Middle School

Honorable Mention: SHANNA SU

Mills E. Godwin High School

Third Place: JORDYN A. WADE

George H. Moody Middle School

Second Place: AMANDA K. RODGERS

Southwest Virginia Governor's School

First Place: ANIRUDH SARASWATHULA

Thomas Jefferson High School for Science and Technology

#### COMPUTER SCIENCE

Honorable Mention: ANDREW C. CASEY

Central Virginia Governor's School

Honorable Mention: MICHAEL R. LEVET

Deep Run High School

Honorable Mention: ANDREW E. VITKUS

George H. Moody Middle School

Third Place: BENJAMIN M. ROBLE

Mathematics and Science High School at Clover Hill

Second Place: LAWRENCE TALEJ

Deep Run High School

First Place: BRIAN K. SEAL, JR.

Deep Run High School

#### CONSUMER SCIENCE A

Honorable Mention: DEVAN M. BITTINGER

Hanover High School

Honorable Mention: KIMBERLY D. CASTLEMAN AND

MACKENZIE G.NEWMAN

Deep Run High School

Honorable Mention: ARIELLE R. EFFRON

Mills E. Godwin High School

Third Place: JOSEPH M. DAMRON III

Mountain Vista Governor's School

Second Place: TARA R. DEAN

Southwest Virginia Governor's School

First Place: LINDSAY A. BYRUM

Chesapeake Bay Governor's School

#### CONSUMER SCIENCE B

Honorable Mention: KATHERINE B. MODLY

Mountain Vista Governor's School

Honorable Mention: ERIN R. VEASEY

Southwest Virginia Governor's School

Honorable Mention: JOSHUA J. WHITE

Shenandoah Valley Governor's School

Third Place: COURTNEY M. SIMS

Shenandoah Valley Governor's School

Second Place: PETER STEELE

Shenandoah Valley Governor's School

First Place: HANNAH M. MEEKS

Hanover High School

#### EARTH AND SPACE SCIENCE

Honorable Mention: NATHANIEL T. BURKHOLDER

Shenandoah Valley Governor's School

Honorable Mention: HANA-MAY EADEH

George H. Moody Middle School

Third Place: STUART D. GEIPEL

George H. Moody Middle School

Second Place: WILLIAM B. RIORDAN

Central Virginia Governor's School

First Place: BRANDEN T. KATONA

Mills E. Godwin High School

## 116 VIRGINIA JOURNAL OF SCIENCE

ENGINEERING A

Honorable Mention: KATHARINE A. GRAHAM

George H. Moody Middle School

Honorable Mention: MAX S. NEWMAN

Mills E. Godwin High School

Honorable Mention: MARY E. SEALS

Central Virginia Governor's School

Third Place: MICHELLE A. KENNEDY

Hanover High School

Second Place: CHRISTOPHER M. WERTMAN

Shenandoah Valley Governor's School

First Place: JOY E. LEE

Thomas Jefferson High School for Science and Technology

ENVIRONMENTAL SCIENCE A

Honorable Mention: REID A. BARDEN

George H. Moody Middle School

Honorable Mention: DICKSON R. BARRY

Patrick Henry High School

Honorable Mention: KATHERINE D. BAUMAN

H. B. Woodlawn

Third Place: KATHERINE J. ADAMS

Chesapeake Bay Governor's School

Second Place: KYLE F. ALLWINE

Chesapeake Bay Governor's School

First Place: MICHAEL A. BUGAS
Fort Defiance High School

ENVIRONMENTAL SCIENCE B

Honorable Mention: GRIFFIN Q. HUNDLEY

Hanover High School

Honorable Mention: GUFRAN H. JARRAR AND CARRIE D. CARDONA

Chesapeake Bay Governor's School

Honorable Mention: SARAH V. LIU

Roanoke Valley Governor's School

Third Place: RUTH W. HEDBERG

Chesapeake Bay Governor's School

Second Place: SAMANTHA L. FLOYD

Chesapeake Bay Governor's School

First Place: GREGORY D. DORSEY

Chesapeake Bay Governor's School

ENVIRONMENTAL SCIENCE C

Honorable Mention: ELIZABETH MCDONALD

Williamsburg Middle School

Honorable Mention: MARK D. MISCH AND JEFFREY C. DITMER, JR.

Chesapeake Bay Governor's School

Honorable Mention: BRIAN P. MURPHY

Chesapeake Bay Governor's School

Third Place: MITHCHELL J. OLIVER

Mountain Vista Governor's School

Second Place: HILLARY D. MAY AND CALEB S. SMITH

Chesapeake Bay Governor's School

First Place: SARAH G. MURPHY

George H. Moody Middle School

ENVIRONMENTAL SCIENCE D

Honorable Mention: NICHOLAS E. ROWE

Chesapeake Bay Governor's School

Honorable Mention: STEVEN K. THOMPSON

Chesapeake Bay Governor's School

Honorable Mention: MEGAN WALZ

Blacksburg High School

Third Place: ABIGAIL J. SIMON

Home Schooled

Second Place: SARA C. TAYLOR

George H. Moody Middle School

First Place: SETH J. THEUERKAUL

Chesapeake Bay Governor's School

GENETICS AND CELLULAR BIOLOGY

Honorable Mention: SWETHA PASALA

Thomas Jefferson High School for Science and Technology

Honorable Mention: CHELISSE D. PERRY

Chesapeake Bay Governor's School

Honorable Mention: ANDREW M. SHORE

Mathematics and Science High School at Clover Hill

Third Place: CONAN ZHAO

George H. Moody Middle School

Second Place: ALLISON S. REID

Central Virginia Governor's School

First Place: CAROLYN SONG

Mills E. Godwin High School

MATHEMATICS

Honorable Mention: THOMAS J. DELGADO

Mathematics and Science High School at Clover Hill

Third Place: ZACHARY TERNER

Mills E. Godwin High School

Second Place: HUNTER W. LONG

Roanoke Valley Governor's School

## 118 VIRGINIA JOURNAL OF SCIENCE

First Place: SOHINI SENGUPTA

Ocean Lakes High School

#### MEDICINE AND HEALTH A

Honorable Mention: QUINN L. BROGAN

Mills E. Godwin High School

Honorable Mention: COURTNEY O. EGAN

Hermitage High School

Honorable Mention: SANJAY T. KISHORE

Southwest Virginia Governor's School

Third Place: JACK A. BOOTH

George H. Moody Middle School

Second Place: CAITLIN E. DOHERTY

Mills E. Godwin High School

First Place: STEPHANIE A. MARQUEEN

Douglas Freeman High School

#### MEDICINE AND HEALTH B

Honorable Mention: KATHERINE A. RODRIGUEZ

Deep Run High School

Honorable Mention: KATELYN K. ROWLAND

Thomas Jefferson Middle School

Honorable Mention: LESLEY E. SUMMERVILLE

James River High School

Third Place: LEANDER C. UNVERDORBEN

George H. Moody Middle School

Second Place: ASHLEY R. TAYLOR

Southwest Virginia Governor's School

First Place: ANGELA C. MENNA

Mills E. Godwin High School

#### MICROBIOLOGY

Honorable Mention: SEBASTIAN T. COUPE

Thomas Jefferson Middle School

Honorable Mention: SAYANTANEE DAS

George H. Moody Middle School

Honorable Mention: ANNA E. KNIGHT AND CATHERINE F. DWORAK

Thomas Jefferson High School for Science and Technology

Third Place: SAMUEL M. RUBIN

Mills E. Godwin High School

Second Place: SAPIR CACHUM, KATHLEEN ATKATSH AND

MANNA FUJIU

Thomas Jefferson High School for Science and Technology

First Place: SANJAY M.KISHORE

Southwest Virginia Governor's School

#### PHYSICAL SCIENCE

Honorable Mention: NICHOLAS A. PARAISO

George H. Moody Middle School

Honorable Mention: SHRUTI R. RAO

George H. Moody Middle School

Honorable Mention: MADELEINE A. SENDEK

Swanson Middle School

Third Place: LEONARD DUBOVOY

George H. Moody Middle School

Second Place: GRANT S. BROUSSARD

George H. Moody Middle School

First Place: PERRIN L. FALKNER

Swanson Middle School

#### **PHYSICS**

Honorable Mention: LAKSHMI BODAPATI

George H. Moody Middle School

Honorable Mention: MADELINE L. BOTTICELLO

Yorktown High School

Honorable Mention: CLAYTON M. GEIPEL

Mills E. Godwin High School

Third Place: BRANDON M. BUNCHER

George H. Moody Middle School

Second Place: SAMUEL C. PASSAGLIA

Washington-Lee High School

First Place: MUKARRAM AHMAD

Mathematics and Science High School at Clover Hill

#### **PSYCHOLOGY - GENERAL**

Honorable Mention: SAMUEL A. AKERS

George H. Moody Middle School

Honorable Mention: ADITYA P. MOTHADAKA

Mills E. Godwin High School

Honorable Mention: MOLLY L. ROSS

Shenandoah Valley Governor's School

Third Place: HALEY T. SQUIER

Shenandoah Valley Governor's School

Second Place: ERIK R. SIMONSEN

Shenandoah Valley Governor's School

First Place: COURTNEY L. YANCEY

Shenandoah Valley Governor's School

#### **PSYCHOLOGY - LEARNING & PERCEPTION A**

Honorable Mention: ALEXANDER ALTHOFF AND CHELSEA A. MILLS

Deep Run High School

Honorable Mention: INDAY J. BARAHONA

Yorktown High School

Honorable Mention: BRANDY D. HENCE

Chesapeake Bay Governor's School

Third Place: SIBLEY A. BROWN

Southwest Virginia Governor's School

Second Place: JACKSON R. COLVER

Mills E. Godwin High School

First Place: KATHERINE L. AGNEW

Central Virginia Governor's School

#### **PSYCHOLOGY - LEARNING & PERCEPTION B**

Honorable Mention: ARAVIND MENON

Deep Run High School

Honorable Mention: ANA O'HARROW

Yorktown High School

Honorable Mention: SCOTTIE M. SMITH

Deep Run High School

Third Place: JULIA G. SHRECKHISE

Shenandoah Valley Governor's School

Second Place: CARLA M. SPENCE

Thomas Jefferson Middle School

First Place: SONIA PHENE

Washington-Lee High School

#### **PSYCHOLOGY - SOCIAL**

Honorable Mention: CHRISTOPHER D. KIME

Yorktown High School

Honorable Mention: SAMANTHA F. SPYTEK AND LARA L. SIERRA

Gunston Middle School

Honorable Mention: AMELIA J. TYLER

Southwest Virginia Governor's School

Third Place: PAUL W. DUCKWORTH

Mills E. Godwin High School

Second Place: WENHAO LU

Mills E. Godwin High School

First Place: SARA BOURDOUANE

Thomas Jefferson Middle School

#### **STATISTICS**

Honorable Mention: WILLIAM J. LUXHOJ AND JACOB L. CHAMBON

Deep Run High School

Third Place: TODD W. PHILLIPS

Mills E. Godwin High School

Second Place: ANDREW R. HOUSE

Deep Run High School

First Place: KATHERINE C. LARSON

Mills E. Godwin High School

#### **ZOOLOGY**

Honorable Mention: LAUREN E. BENNETT

George H. Moody Middle School

Honorable Mention: BRADY K. BROWN

George H. Moody Middle School

Honorable Mention: KATHRYN A. KINGSBURY AND ART T. KULATTI

Thomas Jefferson High School for Science and Technology

Third Place: EMILIA R. SENS

Washington-Lee High School

Second Place: SUSAN M. HASTINGS AND MEGHAN R. KELLY

Thomas Jefferson High School for Science and Technology

First Place: ALEXANDER M. KIM

Thomas Jefferson High School for Science and Technology

## **SPECIAL AWARDS**

Botany Section Award, given by the Botany Section of the VAS, to the best paper on a botanical subject. (\$100.00)

MADHURA V. CHITNAVIS Roanoke Valley Governor's School

Speleological Society Award given to the best paper addressing karst or topics related to speleology given by the Richmond Area Speleological Society. (\$50.00)

HANA-MAY EADEH

George H. Moody Middle School

Mathematics Award for the paper that evidences the most significant contribution in the field of Mathematics. (\$200.00)

SOHINI SENGUPTA Ocean Lakes High School

Statistics Award for the paper that evidences the most significant contribution in the field of Statistics. (\$200.00)

KATHERINE C. LARSON Mills E. Godwin High School

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Smith Shadomy Infectious Disease Award in honor and memory of Dr. Smith Shadomy given by the Virginia Chapter of the National Foundation of Infectious Diseases. (\$50.00)

GREGORY THOMPSON Central Virginia Governor's School KATHERINE E. BAUMANN George H. Moody Middle School

Roscoe Hughes Award for the best paper in the field of Cellular Biology. (\$150.00)

CHELISSE PERRY
Chesapeake Bay Governor's School

Rodney C. Berry Chemistry Award for the paper that evidences the most significant contribution in the field of chemistry. (\$150.00)

PRASANNA G. JOSHI Mills E. Godwin High School

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry will be given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life. (\$150, \$100)

Second Place EMILY SPILLER

George H. Moody Middle School

First Place AMANDA K. RODGERS

Southwest Virginia Governor's School

Catesby Jones - Russell J. Rowlett Award for the Best Research Paper of the Year. (\$100.00)

JOY LEE

Thomas Jefferson High School for Science and Technology

Virginia Sea Grant College Program Award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research. (\$50.00)

SETH THEUERKAUF

Chesapeake Bay Governor's School

American Cancer Society Award - This award is to recognize outstanding science papers related to cancer research. A certificate to each and to 1st place - \$100, honorable mentions - \$50. These awards are funded by the American Cancer Society (Virginia Council).

Honorable Mention AMANDA K. RODGERS

Southwest Virginia Governor's School

Honorable Mention CONAN ZHAO

George H. Moody Middle School

First Place PAUAN G. GUDIMETTA

Deep Run High School

The Gamma Sigma Delta Award (Agriculture). Presented by the VPI & SU Chapter of the Honor Society of Agriculture. This award is presented in recognition of excellence in research dealing with application of new technologies and/or concepts in agriculture forestry, or veterinary medicine. (\$100)

TIAN ZHOU Blacksburg High School

Dominion - W.W. Berry Award. This award is given by Dominion Virginia Power in honor of Mr. W. W. Berry who was a past Chairman of the Board of VA Power. This award of a \$500.00 Savings Bond will be presented to the best engineering paper. The winners must see the Director by the stage after the awards ceremony.

JOY E. LEE

Thomas Jefferson High School for Science and Technology

The Joyce K. Peterson Award is presented for the outstanding paper by a middle school student. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools. (\$50)

CONAN ZHAO

George H. Moody Middle School

The Ann M. Hancock Award - This award is given to the best paper in genetics and is given in memory of Anne Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee. (\$100)

CAROLYN SONG

Mills E. Godwin High School

Dorothy Knowlton Award - This award is given to the best paper in the Consumer Science section(s) and is given in honor of Dorothy Knowlton, former Science Coordinator of Arlington County Schools. (\$50)

BRITTANY COOK

Southwest Virginia Governor's School

VABE Award - This award is presented by the Virginia Association of Biology Educators and is given for outstanding research in the Zoology section. (\$100)

ALEXANDER KIM

Thomas Jefferson High School for Science and Technology

Virginia Museum of Natural History Award - Presented by the Friends of the Virginia Museum of Natural History in recognition of significant contribution in the study and interpretation of Virginia's Natural Heritage. The winner will receive \$100

SETH THEUERKAUF Chesapeake Bay Governor's School Trip to AJAS - AAAS Meeting for two students for presenting outstanding papers. The 2009 meeting will be held in Feb.in San Diego.

Winner BRANDEN T. KATONA

Mills E. Godwin High School

Winner PRASANNA G. JOSHI

Mills E. Godwin High School

Honorary Membership - AAAS given to two students.

PRAKRITI VERMA Grafton High School TARA ADISHESAN Ramana Academy

Honorary Membership - VAS given to a student.

ELIZABETH GENTRY Atlee High School

Bethel High School Scholarship - This \$1,000 Scholarship Award comes from the interest earned from a \$10,000 endowment contributed by the students of Bethel High School, Hampton, Va., over a two year period. This award is based on both the students presentation and paper.

KATHERINE AGNEW
Central Virginia Governor's School

Henry MacKenzie Environmental Scholarship - This \$5,000 scholarship will be awarded to the student whose paper evidences the most significant contribution in the field of Environmental Science dealing with the James River Basin and Chesapeake Bay. The Virginia Endowment and VJAS offer this scholarship in tribute to the outstanding and generous services of Judge Henry W. MacKenzie, Jr., one of the founding directors who has a great interest in the James River and the Chesapeake Bay.

SETH THEUERKAUF Chesapeake Bay Governor's School

Frances and Sydney Lewis Environmental Scholarship: A \$14,000 scholarship (\$3,500 per year for four years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This scholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental Endowment.

ALEXANDER KIM

Thomas Jefferson High School for Science and Technology

E.C.L. Miller Science Teacher of the Year Award is given to an outstanding science teacher. An all-expense-paid trip to the next VAST meeting held in November.

MARIVIC MITCHELL
George H. Moody Middle School

VJAS Distinguished Service Award, most prestigious award given by the VJAS, is presented to a person for exceptionally outstanding service to the VJAS.

PAM GENTRY

## 2009-2010 VJAS Officers

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M. Leroy Spearman

M. Leroy Spearman, a research scientist at the National Aeronautics and Space Administration Langley Research Center, has contributed to the advancement of scientific research and science education in Virginia and in his discipline throughout his career. Through his research on airfoils, surface flow, and materials and his management of wind tunnel facility projects, he has become a national leader. He has mentored countless graduate students and managed their projects. For several decades. he has enthusiastically and tirelessly served the Academy as Chair and Program Chair of the Academy's Aeronautics and Aerospace Sciences Section.

Leroy Spearman retired from NACA/NASA December 31,2004 after over 60 years of government service. He earned a B.S. degree in Aeronautical Engineering from Auburn University, 1943, and began his government service at Langley Field, VA in March 1944. At the time of retirement he was an aerospace technologist in the Systems Analysis Branch, Aerospace Systems, Concepts & Analysis Competency at the NASA-Langley Research Center, Hampton, VA where he was involved in assessing advanced vehicles including civil transports, hypersonic vehicles, various types of missiles and new innovations. He is recognized as an authority in the fields of aerodynamics, stability and control, and performance of aircraft, spacecraft and missiles. Leroy has conducted wind tunnel research investigations throughout the subsonic, transonic and supersonic speed ranges.

Leroy began his career in 1944 with NACA at what was then the Langley Memorial Aeronautical Laboratory. He was assigned to the Atmospheric Wind Tunnel where he made some of the first tests of swept wings in the US.

He was transferred to the new 7 x 10-foot tunnels in 1946 where he continued his study of swept wings. He conducted some of the earliest transonic tests in the US using the transonic-bump technique. These tests revealed, for the first time, some of the

aerodynamic phenomena to be encountered in flight at supersonic speeds. These tests included a model of what was to become the first airplane to exceed the speed of sound, the Bell X-1.

In 1948, he was transferred to the new 4 x 4-foot supersonic pressure tunnel where he extended his aerodynamic studies to supersonic speeds. There he was responsible for stability and control studies that had an impact on essentially every supersonic aircraft and missile built in the US. He made the first supersonic tests of the variable sweep wing concept, canard airplane and missile configurations, and some of the earliest tests of supersonic transport concepts.

In 1963, he began to conduct research studies including wind-tunnel tests that were designed to assess the status of foreign technology. Over the years these studies have had a significant effect on the direction taken by some US programs.

In 1974, Spearman was assigned to the High-Speed Research Division Office as Chief Scientist for Military and Foreign Technology. He was reassigned in 1979 to the Aeronautical Systems Division as a Senior Technical Specialist where he participated in the development and assessment of a variety of advanced vehicles.

In addition to his significant contributions to research. Mr. Spearman has participated as a mentor in the New Horizons Regional Education Center program for talented-and-gifted high-school students and in the NASA Langley Virginia Governor's School Program. For several years he has instructed, guided, and encouraged those students who are looking toward a career in engineering. He also promoted interest in math and science as a guest teacher in local schools.

Leroy is a Fellow of the American Institute of Aeronautics and Astronautics. He is a member of the Air Force Association, the Auburn University Alumni Engineering Council and the Virginia Academy of Science. He has been honored by all of these groups and has received a number of NASA awards as well.

Leroy Spearman is credited with authoring over 316 technical publications. He continues to work with NASA as an unpaid Distinguished Research Associate, and continues to author papers for technical societies.



## **Donald Allen Whitney**

Dr. Donald Whitney, a physicist and Dean of the Graduate School at Hampton University, has contributed to the advancement of scientific research, science education, and research and education management in Virginia and in his disciplines throughout his career. For several years he has enthusiastically and tirelessly served the Academy as President and member of Council and as Chair of Local Arrangements for several Academy annual meetings at Hampton University.

Don, Dean of the Graduate College and Associate Professor of Physics at Hampton University. served as 84th President of the Virginia Academy of Science (2006-07). A long time member of the Academy's Astronomy, Mathematics and Physics Section, he has served as the Section's Chair, Secretary and several terms as Editor. His Academy offices include President-Elect. Vice President, Secretary, and Treasurer. Don chaired the Local Arrangements Committees for 80th and 86 Annual Meetings (2000-02 and 2006-08), was Program Chair for the 83rd Annual Meeting at James Madison University (2005), and currently serves on the Finance and Nominations committees.

Don's first of several presentations to the Academy was a paper on solar energy measurements in 1982. Characteristic of much of his career as a science educator, many other papers would be co-authored with student co-investigators. As a scientist and research and academic administrator, Hampton University graduate and undergraduate students would also benefit from \$2 million in grants, for which Dr. Whitney was Principal Investigator or Project Director, from the National Science Foundation, Sherman Fairchild Foundation, U.S. Department of Education, National Aeronautic and Space Administration, and Battelle Pacific Northwest Laboratory.

Don's dedication to science education extends as well to middle and secondary students and their teachers through workshops, lectures, and demonstrations for city and county schools and judging science fairs and local science fair projects. For over twenty years, Don has served as a Judge for the Annual Meetings of the Virginia Junior Academy of Science.

Dr. Whitney's research interests are in laser physics, environmental and atmospheric sciences, and low temperature solid state physics. His studies have been published in American Institute of Physics Proceedings, Bulletin of the American Physical Society, Progress in Solar Energy, Solid State Communications, and Physical Review.

At Hampton University, he has served on more than fifty governance committees, often as chair. Hampton recognized Don's leadership early, naming him the university's first Assistant, and then Associate, Dean. As a consultant, he has contributed to physics education and curriculum design for the U.S. Air Force Academy, University of Dallas,

NASA-Langley Research Center, National Science Technology Society. U.S. Department of Education, and as a reviewer for several textbook publishers. For several years he has worked on physics education standards of the MCAT and NTE for the Educational Testing Service.

Donald Allen Whitney holds a B.S. in Physics from the University of Scranton (1969) and the Ph.D. in Physics from the University of Virginia (1977). He was an NDFA Fellow, Eli Lilly Post-Doctoral Fellow, and an American Society for Engineeriniz Research Fellow. A member of Sigma Xi National Honor Society, Dr. Whitney has been listed in Who Who in the South and Southwest and Who s' Who Among Americas' leachers.



#### Susan P. Booth

Susan Booth has contributed to the advancement of science education in Virginia as a teacher and teacher educator throughout her career. For several years she has enthusiastically and tirelessly served the Academy through its Virginia Junior Academy of Science Committee, as Director of the Virginia Junior Academy of Science, and by her work with the American Junior Academy of Science and similar organizations.

Susan P. Booth has been Director of the Virginia Junior Academy of Science since 1999 and Executive Director of the Virginia Association of Science Teachers since 2000. Susan has taught at Kecoughtan High School since 1986, where she was named "Biology Teacher of the Year" and "Teacher of the Year," and at Lee-Davis High School (1984-86) and .I.E.J. Moore High School (1983-84). From 1991-97, she was the Newport News School System's Science Specialist, K-12.

Susan's enduring interest in VJAS includes decades of service on the VAS Junior Academy Committee and later as VJAS Assistant Director. In 1991 she was honored with VMS Science Club Sponsor Award. The breadth and depth of her many contributions to science education has also been recognized in the Outstanding Volunteer Award of the Virginia Association of Science Teachers (1992), the Distinguished Service Award of the Tidewater Science Congress (1994), and the Equity Award of the National Association of Biology Teachers (1996).

A consultant for International Science and Engineering Fairs, Susan Booth has presented papers and conducted teacher workshops for the Tidewater Science Congress, V-Quest (Virginia Department of Education), the Virginia Association of Science Teachers. and NASA Langley Research Center. She has published articles in the *Journal of Virginia Science Education* and *The Daily Press* and has been awarded grants from the Virginia Department of Education, the Virginia Commission for the Arts, and the American Association for the Advancement of Science.

Currently, Susan also serves on the Tidewater Science Congress Advisory Board and the Board of Directors of the Virginia Association for Supervision and Curriculum

Development. She has been a National USA/USSR Educator (1990) and a NASA Project Stars/V-Quest Initiative Teacher/Researcher (1994). In service to her community she was Hampton's Clean City Coordinator and School Pride in Action Coordinator; Susan was honored by the City of Hampton with its Outstanding Volunteer Award (1993).

Susan majored in Biology-General Science at Mary Washington College (B.S., 1983) and earned her M.A. in K-12 Supervision (1992) and Ed.S. in K-12 Administration (1993) from George Washington University. Her Virginia certifications include General Science and Biology, Elementary School Principal, Middle School Principal, and High School Principal.

A member of Phi Delta Kappa National Honor Society, Susan is also a member of the Mathematics/Science Coaltion-Region 2, the Virginia Science Resource Network, the National Association of Biology Teachers, and she is a an Academic Reviewer for the Virginia Department of Education. Susan Booth has been also been named to several editions of Who Who Among Americas' Teachers.

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#### THE VIRGINIA JOURNAL OF SCIENCE

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# VIRGINIA JOURNAL OF SCIENCE

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# First Records of Hypleurochilus geminatus and Centropristis philadelphica from Chesapeake Bay Aimee D. Halvorson<sup>1</sup>, Virginia Institute of Marine Science, Department of Fisheries Science, PO Box 1346, Gloucester Point, Virginia 23062, USA

#### **ABSTRACT**

During the fall of 2007, Centropristis philadelphica (rock seabass) and Hypleurochilus geminatus (crested blenny) were collected from Chesapeake Bay. These captures are significant as they represent the first substantiated record of C. philadelphica from Chesapeake Bay and only the second and third validated records of H. geminatus. Additionally, the first record of H. geminatus from Chesapeake Bay was only recently recognized since the specimen had been previously misidentified as Parablennius marmoreus (seaweed blenny). The collection of seven individuals of H. geminatus in 2007, from two locations, indicates that the species may be resident within the Chesapeake Bay estuary.

#### INTRODUCTION

The Chesapeake Bay, an ecotone between the Atlantic Ocean and the rivers of Maryland and Virginia, experiences extreme seasonal temperature changes and contains a range of habitats. Species richness is typical of such ecological systems and is evident by the estuary's diverse and dynamic fish fauna, which includes permanent residents, spawning migrants, and seasonal visitors (Murdy et al. 1997). The fish fauna of Chesapeake Bay has been surveyed extensively since the early 1900's (Hildebrand and Schroeder 1928; Massman 1962; Massman and Mansueti 1963; Musick 1972; Murdy et al. 1997) yet warmwater species uncommon to the estuary continue to be encountered (Halvorson 2007). Two such species, *Centropristis philadelphica* (rock seabass) and *Hypleurochilus geminatus* (crested blenny), were collected in Chesapeake Bay during the fall of 2007 by the Virginia Institute of Marine Science (VIMS) Juvenile Fish and Blue Crab Trawl Survey.

#### MATERIALS AND METHODS

Five-minute bottom tows were conducted in lower Chesapeake Bay with a 9.14 m otter trawl (38.11 mm stretched mesh body, 6.35 mm cod-end liner, and a tickler chain) off the 8.5 m R/V Fish Hawk. Fish were identified and measured to the nearest mm (total length for *H. geminatus* and total length centerline for *C. philadelphica*). Voucher specimens were deposited in the Ichthyological Collection, Virginia Institute of Marine Science, Gloucester Point, Virginia (*H. geminatus*-VIMS 11776, *C. philadelphica*-VIMS 11979). Hydrological measurements (water temperature, salinity) were taken with a YSI 600Q (YSI Incorporated, Yellow Springs, Ohio).

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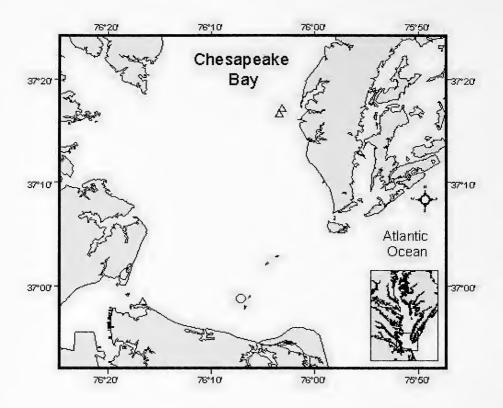


FIGURE 1. Collection locations of Centropristis philadelphica ( $\circ$ ) in 2007 and Hypleurochilus geminatus ( $\Delta$ ) in 1993 and 2007 in Chesapeake Bay.

#### RESULTS

On September 6<sup>th</sup>, 2007, five individuals of *H. geminatus* (39-78 mm) were captured in Chesapeake Bay at 37°17.13'N, 76° 03.11'W, near Cape Charles, Virginia (Figure 1; Table 1). Water depth at this station was 7 m and the bottom water temperature and salinity were 26.59°C and 23.64‰, respectively. Two additional specimens (34-37 mm) were collected on November 14<sup>th</sup>, 2007, at 36°58.43'N, 76°16.59'W, near the entrance to Hampton Roads, in 5.5 m of water (Figure 1; Table 1). The bottom water temperature was 13.63°C and bottom salinity was 22.79‰.

A single specimen of *C. philadelphica* (210 mm) was collected November 5<sup>th</sup>, 2007 at 36°58.76'N, 76°07.16'W, approximately 1 km upstream of the first tunnel of the Chesapeake Bay Bridge-Tunnel (Figure 1; Table 1). Water depth was 13.4 m and the bottom water temperature and salinity were 17.22°C and 24.54%, respectively.

#### DISCUSSION

The crested blenny (*Hypleurochilus geminatus*) is a subtropical species often found in association with oyster reefs, shell bottoms (Dahlberg 1972; Crabtree and Middaugh 1982; Lehnert and Allen 2002), and marine growths attached to pilings and rocks (Hildebrand and Cable 1938). They feed on free swimming organisms as well as sessile

Table 1. Table of species showing the number of specimens, year collected, and collection location (latitude and longitude).

Species	Year Collected	Number of specimens	Latitude	Longitude
Centropristis philadelphica	2007	1	36°58.76N	76°07.16W
Hypleurochilus geminatus (reported by Murdy et al. 1997 as Parablennius marmoreus)	1993	1	37°16.63N	76°03.43W
Hypleurochilus geminatus	2007	5	37°17.13N	76°03.11W
Hypleurochilus geminatus	2007	2	36°58.43N	76°16.59W

growths (Hildebrand and Cable 1938), with their diets primarily consisting of crustaceans and algae, followed by hydroids and polychaetes (Lindquist and Chandler 1978; Lindquist and Dillaman 1986). Hildebrand and Cable (1938) determined that North Carolina specimens of *H. geminatus* spawn from May to September and the larvae are mainly surface dwelling until 10-15 mm in length, at which time they change their habitat preference. The largest fish collected in their study was a 72 mm male, with the largest female measuring 58 mm (Hildebrand and Cable 1938).

Although the range of H. geminatus encompasses the waters of New Jersey to the eastern central coast of Florida (Williams 2002), the only collections north of North Carolina have occurred sporadically off New Jersey (Fowler 1914; Allen et al. 1978; Able 1992; Able and Fahay 1998). Hypleurochilus geminatus was not reported in earlier studies of Virginia waters, including Chesapeake Bay and its tributaries (Hildebrand and Schroeder 1928; Massman 1962; Massman and Mansueti 1963; Musick 1972; Murdy et al. 1997) and the seaside coasts and inlets (Schwartz 1961; Richards and Castagna 1970; Cowan and Birdsong 1985; Norcross and Hata 1990; Layman 2000). Ditty et al. (2005) erroneously reported that Hildebrand and Cable (1938) obtained larvae of H. geminatus from Chesapeake Bay. Ongoing baywide surveys, including the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP) (James Gartland, Virginia Institute of Marine Science, Gloucester Point, Virginia, personal communication) and the Chesapeake Bay Fishery-Independent Multispecies Survey (CHESFIMS) (Miller and Loewensteiner 2008), have yet to encounter this species, nor do specimens from Chesapeake Bay exist in the VIMS Ichthyological Collection or the U.S. National Museum (USNM) fish collection (L. Palmer, Smithsonian Institution, pers. comm.).

The captures in 2007 are not the first records of *H. geminatus* collected from Chesapeake Bay. Murdy et al. (1997) reported a single specimen of *Parablennius marmoreus* (seaweed blenny) captured in June 1993 (VIMS specimen 09086). Upon

further evaluation in 2007, it was determined that this specimen had been misidentified and is indeed *H. geminatus*. Interestingly, this specimen was collected at 37°16.63'N, 76°03.43'W (Figure 1; Table 1), within 1 km from the location where five individuals were collected in September 2007. The collection of a single misidentified *H. geminatus* in 1993 is the first documented occurrence of this species in Chesapeake Bay and the subsequent capture of seven individuals during 2007 indicates that not only has this species extended its range to include the estuary, but that an established population might exist off Cape Charles, VA.

The smallest member of the genus Centropristis, C. philadelphica is a fast growing, short-lived species (Link 1980) that attains a maximum length of 300 mm (Heemstra et al. 2002). This protogynous hermaphrodite inhabits a range of depths over various substrates, including hard bottoms, rocky reefs, and the preferred softer mud bottoms (Miller 1959; Link 1980). Spawning occurs offshore between February and July (peak April-May) off North Carolina (Link 1980) and from late March to May in the Gulf of Mexico (Miller 1959). Ross et al. (1989) described C. philadelphica as a "euryphagic benthic carnivore" and their study of Gulf of Mexico specimens found a diet dominated by shrimps, crabs, mysids, and fishes, agreeing with Links' (1980) findings that crustaceans, fishes, and mollusks were the most frequent prey.

The range of *C. philadelphica* includes Cape Henry, Virginia, to Palm Beach, Florida, as well as the Gulf of Mexico (Miller 1959; Heemstra et al. 2002). *Centropristis philadelphica* was not reported in earlier studies of Chesapeake Bay and its tributaries (Hildebrand and Schroeder 1928; Massman 1962; Massman and Mansueti 1963; Musick 1972; Murdy et al. 1997) nor the Virginia seaside coasts and inlets (Schwartz 1961; Richards and Castagna 1970; Cowan and Birdsong 1985; Norcross and Hata 1990; Layman 2000). Ongoing baywide surveys including the ChesMMAP (James Gartland, Virginia Institute of Marine Science, Gloucester Point, Virginia, personal communication) and the CHESFIMS (Miller and Loewensteiner 2008) have yet to encounter this species, nor are there specimens from Chesapeake Bay in the VIMS Ichthyological Collection or the U. S. National Museum (USNM) fish collection (L. Palmer, Smithsonian Institution, pers. comm.).

The individual collected in November 2007 represents the first substantiated record for *C. philadelphica* from Chesapeake Bay. The Northeast Fisheries Science Center (NEFSC) trawl survey's most northerly validated record of *C. philadelphica* is a 100 mm standard length specimen from 37°28'N, 74°25'W, approximately 100 km east of Parramore Island, Virginia, in the Atlantic Ocean (William Kramer, NOAA Fisheries Service, Woods Hole, Massachusetts, personal communication). Both of these occurrences are slightly north of the published northern range boundary of Cape Henry, Virginia.

Nearly twenty years ago, Kennedy (1990) predicted that climate change would cause "poleward estuaries to resemble neighboring estuaries that are located in the direction of the equator." As such, he stated that Chesapeake Bay could become as warm as southeast Atlantic coast estuaries and that warmwater or subtropical species would move north from these neighboring estuaries and occupy Chesapeake Bay (Kennedy 1990). Interestingly, the VIMS Juvenile Fish and Blue Crab Trawl Survey, which has sampled Chesapeake Bay and its tributaries since 1955, has recently documented an increase in the diversity of Chesapeake Bay warmwater fishes. Three

previously unsubstantiated warmwater species were collected from the estuary during 2004 and 2005: Trachinocephalus myops (snakefish), Citharichthys macrops (spotted whiff), and Mullus auratus (red goatfish) (Halvorson 2007). In addition, the survey collected its first verified specimen of C. philadelphica and seven individuals of H. geminatus in 2007. These data are not only significant for monitoring such phenomena as climate change, but also for updating field guides; these substantiated reports from 2004-2007 include four species that have yet to be profiled in "Fishes of Chesapeake Bay" (Murdy et al. 1997) and documents range extensions for three species in "A Field Guide to Atlantic Coast Fishes" (Robins et al. 1986).

The collection of multiple unsubstantiated species also illustrates the importance of voucher specimens, whether to re-evaluate the identification of an individual or to verify that a species was indeed collected and documented correctly. Scientists should be aware that the fish fauna of Chesapeake Bay is dynamic and that vigilance is necessary to recognize uncommon species, many which appear similar to known residents. The knowledge of additional species (e.g. H. geminatus) inhabiting Chesapeake Bay is essential when studying ecological interactions such as predator-prey relationships and competition. The information gained from these collections demonstrates the importance of long-term monitoring surveys and their usefulness in documenting changes in marine and estuarine environments.

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# Phytoplankton Blooms: Their Occurrence and Composition Within Virginia's Tidal Tributaries

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#### **ABSTRACT**

Sporadic algal bloom development within a 10 year monitoring program in Virginia tidal tributaries of Chesapeake Bay is reviewed. These blooms were common events, characteristically producing a color signature to the surface water, typically short lived, occurring mainly from spring into autumn throughout different salinity regions of these rivers, and were produced primarily by dinoflagellates. The abundance threshold levels that would identify bloom status from a non-bloom presence were species specific, varied with the taxon's cell size, and ranged from ca. 10 to 10<sup>4</sup> cells mL<sup>-1</sup>. Among the most consistent sporadic bloom producers were the dinoflagellates Akashiwo sanguinea, Cochlodinium polykrikoides, Heterocapsa rotundata, Heterocapsa triquetra, Karlodinium veneficum, Prorocentrum minimum, Scrippsiella trochoidea, the cyanobacterium Microcystis aeruginosa, and two categories containing several species of often unidentified Gymnodinium spp. and Gyrodinium spp. Additional bloom producers within these tributaries are also discussed.

Keywords: Virginia, rivers, phytoplankton, blooms, Chesapeake Bay.

#### INTRODUCTION

Algal blooms occur in freshwater habitats, estuaries, the world oceans, and are natural phenomena (Anderson et al., 2002). The term "algal bloom" refers to high concentrations of one or more algal species, and generally implies visual recognition of this development by color enhancement in the water column due to pigments contained in the algal cells. These colors may vary due to the different types and amount of pigments within the cells of the bloom producing species. Algal blooms have also been associated with toxic events (e.g. red tides) involving fish and shellfish mortality and human illness (Falconer, 1993; Anderson et al., 2002). Many of these species have been referred to as producing harmful algal blooms (HAB), with concern regarding their apparent increased occurrences in estuaries and oceans world-wide (Smayda, 1990; Hallegraeff, 1993; Anderson et al., 2002; Burkholder et al., 2005). In many of the toxin producing species the bloom designation becomes a secondary factor to the presence of a toxin and established toxin threshold levels of concern (Rensel and

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Whyte, 2003). Within the Chesapeake Bay estuarine system a variety of potentially harmful species and bloom producers have been identified and many of these are common constituents of the river flora in Virginia (Marshall, 1996; Marshall et al., 2005, 2008a). The presence alone of these recognized toxic species does not indicate they will cause a serious impact to the health status of these waters. Cell concentrations may not reach the abundance levels required for significant levels of toxin production that would have an environmental impact (Smayda, 1997; Marcaillou et al., 2005), or these may be non-toxin producing strains of the toxic species (Burkholder et al., 2005). However, blooms of both the toxin or non-toxin producing species can deteriorate water quality to the extent that they may impact various indigenous biota (e.g. by reducing oxygen levels, impairing gill function in fish and shellfish).

The environmental impact of an algal bloom would depend on the duration of the bloom, the taxon producing the bloom, and its cell concentrations. However, a wide range of cell concentrations have been associated with bloom status among the phytoplankton components. Paerl (1988) refers to blooms produced by different taxa ranging in abundance from 10<sup>4</sup> to >10<sup>6</sup> cells mL<sup>-1</sup>, whereas Smayda (1990) mentions bloom maxima occurring at sea of 10 cells mL<sup>-1</sup> to >10<sup>4</sup> cells mL<sup>-1</sup>. Kim et al. (1993) identified variable bloom concentrations attributed to several species in the southeastern coastal waters of Korea. They noted low bloom densities of 10<sup>2</sup> to 10<sup>4</sup> cells mt<sup>1</sup> and high bloom densities for particular species ranging from 10<sup>2</sup> to 10<sup>5</sup> cells ml<sup>-1</sup>. These differences are most often influenced by the cell size of the bloom producing species. Many of the smaller nanoplankters would require a greater number of cells to produce a visible bloom signature in the water compared to larger cells and filamentous Kim et al. (1993) subsequently recommended cell volume thresholds for taxa. identifying red tide blooms as 3 X 106 µm3 for nanoplankton and 5 X 106 µm3 for the larger cells of the microplankton. In another approach, Tett (1987) associated general and exceptional bloom events in reference to their chlorophyll concentrations per unit volume of water, with noticeable changes in water discoloration began when levels exceeded 10 mg Chl m<sup>-3</sup>. The larger exceptional blooms had values greater than 100 Species specific criteria have also been used; for instance the Commonwealth of Virginia established a chlorophyll level of 27.5 µg L<sup>-1</sup> (27.5 mg Chl m<sup>-3</sup>) and 50,000 cells ml<sup>-1</sup> as bloom criteria for Microcystis aeruginosa a potential toxin producer.

A particular taxon may also have cell concentrations and biomass lower than that of other taxa within the water column, but still represent a major development in its annual productivity, yet not dominating the algal assemblage (Parker, 1987; Smayda, 1997). This is frequently noted in annual monitoring programs where background flora of usual low abundance, may seasonally achieve a modest, but often a short-lived period of high abundance, with their concentration levels and degree of color enhancement to the water lower than other more abundant or larger taxa. Reference to these abundance peaks represent an alternate method of describing bloom status that may or may not include a color signature to the water column, but relate to the seasonal population dynamics that is species specific.

Conditions associated with the inception and duration of seasonal blooms include a variety of environmental factors: e.g. concentrations of nutrients (e.g. nitrogen, phosphorus, silicon, etc.), temperature, salinity, light availability, river flow, cloud cover, grazing pressure, among other factors (Pratt, 1965; Riley, 1967; Tett, 1987;

Smayda, 1990; Keller et al., 1999, 2001; Glibert et al., 2001; Anderson et al., 2002; Iriarte and Purdie, 2004). Seasonal blooms of short or long duration are determined by various combinations of these conditions and their influence on the composition and abundance of the flora and potential bloom producers. These bloom events may, or may not be associated with foul odors, fish or shellfish mortality, reduced oxygen levels, or human illness. The degree of color enhancement to the water due to bloom development would also vary with the taxon and its abundance over time. Some blooms produce a clearly recognizable color signature in the water, whereas with other taxa the bloom presence will not be clearly visible. In general, blooms occur when one or more species respond to environmental conditions favorable to their increased development beyond their usual abundance levels. Smayda and Reynolds (2001) characterize this response as stochastic, influenced by the characters and traits innate to a species, and their ability to take advantage of prevailing conditions within the water body, and directly respond with increased concentrations.

Seasonal phytoplankton composition for Virginia tidal tributaries and the southern Chesapeake Bay have been recorded routinely by Old Dominion University (ODU) Phytoplankton Analysis Laboratory (ODUPAL) since 1985 (Marshall, 1994; Marshall et al., 2005). Phytoplankton composition and seasonal representation of taxa within the tidal rivers and Chesapeake Bay include a diverse algal representation (>1,400 taxa) and seasonal successional patterns of dominant bloom producers characteristic of temperate regions (Marshall, 1990, 1994, 1995a; Marshall and Nesius, 1996; Marshall and Burchardt, 1998, 2003, 2004a, 2004b, 2005; Marshall et al., 2005, 2009). The objectives of this paper are to provide information on sporadic bloom producing algae in Virginia tidal waters with information regarding the frequency and locations of these bloom events. In addition, cell abundance criteria are provided to formerly classify bloom status for these bloom producers.

#### **METHODS**

The ODUPAL has closely interacted with the Virginia Department of Health Division of Shellfish Sanitation (VDHDSS) and the Virginia Department of Environmental Quality (VDEQ) in providing information on the identification of algal species associated with bloom events in Virginia waters for several decades. In addition, a Virginia program initially designated in 1998 as the *Pfiesteria* Task Force (later renamed the Harmful Algal Bloom Task Force) was established to monitor potentially harmful algal blooms in Virginia waters. With the exception of 2003, routine water samples from this program were taken monthly March-October from 1998, with additional collections taken during any major algal bloom or fish-kill events. These samples were provided to the ODUPAL by VDHDSS and VDEQ for determining species identification and their abundance. Data from these collections through 2008 have been incorporated in this report.

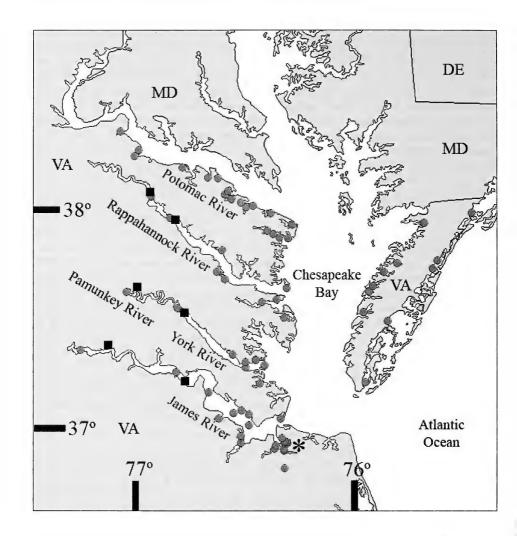


FIGURE 1. Station locations monitored 1998-2008 for algal blooms. ■ = VADEQ Stations, ● = VADH stations, VA = Virginia, MD = Maryland, DE = Delaware, \*location of Elizabeth and Lafayette rivers.

These investigations also included water quality data related to seasonal and sporadic algal blooms, and population trends within the Chesapeake Bay estuarine complex (Marshall and Burchardt, 2004a; Marshall et al., 2006, 2008a, 2009; Nesius et al., 2007). The mean number of stations monitored annually during this period was 78. A total of 4,467 preserved water samples were analyzed during these collections (1998-2008).

The water samples (0.5 or 1.0 L) were taken at the surface (< 1m) and fixed on station with Lugol's solution (2-3 ml). Standard light microscopic protocols were used with the algae examined at 300X and 600X for species identification and cell counts

(Marshall et al., 2005). This protocol was often supplemented with scanning electron microscopy, and more recently using PCR analysis to verify the presence of several potentially harmful species (Marshall et al., 2009). Water quality parameters were determined by the VDEQ and the ODU Department of Chemistry and Biochemistry.

#### RESULTS

A total of 51 tributary and various sub-estuarine sites were identified where algal bloom events occurred, often repeatedly and annually at the same locations. Blooms were recorded at 26 creeks, 17 rivers, and 6 inlet bays in Virginia. Several of these blooms also progressed into lower Chesapeake Bay and to coastal waters along the Virginia Beach shoreline. Among the most common locations were the shoreline inlets, creeks, and waters of the Potomac, York, and Rappahannock rivers, plus a river complex in the lower James River that includes the James, Warwick, Lafayette and Elizabeth rivers (Fig. 1). Using the VDHDSS data base of 1998-2002, 2004-2008), and the VDEQ collections 1998-2008, the number of recorded blooms by 43 taxa ranged from 35 (2002) to 142 (2000) annually. There was a total of 685 blooms identified within the 4,467 samples examined, indicating 15.3% of the water samples contained bloom concentrations of at least one species. The highest number of blooms occurred in 2000 and 2001 which were also years of lower mean river discharge in the rivers of Chesapeake Bay (U.S. Geological Survey monthly stream flow data). During summer and early autumn, major algal development increased in the lower reaches of these rivers during periods of reduced river flow and longer phytoplankton residency time within these rivers (Marshall and Burchardt, 1998, 2003, 2004a, 2004b, 2005).

April through September was the predominant time period for blooms within these tributaries, with the lowest occurrence in December and January. These blooms were generally dominated by dinoflagellates, with the majority of blooms occurring in water temperatures between 18 and 30 °C, salinities of 8 to 18 ppt, and Secchi depths < 1.2 These blooms occurred over a broad range of these parameters, which was indicative of growth responses by a variety of taxa to conditions favoring their increased development. Oxygen concentrations during these blooms were consistently above dystrophic levels (> 4 mg L<sup>-1</sup>). However, no records were kept of oxygen concentrations at these sites throughout the bloom development. Using a 4-year (1998-2001) portion of the VDHDSS tributary station data, Weber and Marshall (2002) noted water quality conditions during bloom events by dinoflagellates classified as Pfiesterialike organisms (PLO). This category included Pfiesteria piscicida, Pfiesteria shumwayae, and several other taxa grouped at that time as morphologically similar under light microscopy (e.g. several Gymnodinium spp. and Gyrodinium spp., plus Cryptoperidiniopsis sp. and Karlodinium veneficum). This category's bloom concentrations and color signatures in the water were associated with the following range of environmental conditions: salinity (8.0-18.4 ppt), temperature (18.0-26.1 °C), chlorophyll a (>16 µg L<sup>-1</sup>), total phosphorus (>0.01 mg L<sup>-1</sup>), TKN (>0.5 mg L<sup>-1</sup>), total dissolved nitrogen (>0.31 mg L<sup>-1</sup>), particulate carbon (>0.25 mg L<sup>-1</sup>), ammonia (>0.04 mg L<sup>-1</sup>), dissolved oxygen (6.7-13.1 mg L<sup>-1</sup>), and Secchi depth (<1.0 m). These parameters were generally similar to conditions throughout the complete data set when dinoflagellate blooms occurred in these tributaries. The concentration levels among the phytoplankton when they imparted a color pattern to the water column varied

considerably between early and later stages of the bloom, as did the color intensity, e.g. higher cell concentrations were often noted along tidal fronts or at near shore locations. There were also temporal differences in the initiation and development of blooms at stations within a river, and of similar events in adjacent rivers. The threshold abundance levels for identifying bloom status varied among the dinoflagellates and were related to their cell size and pigment content. In general, larger cells produced distinct coloration during modest bloom development in contrast to less distinct bloom color enhancement with higher cell concentrations from a smaller size bloom producer. For instance, Akashiwo sanguinea and Cochlodinium polykrikoides have larger cell sizes and pigment concentration, with lower threshold levels for bloom status than species with smaller cell sized cells (e.g. Microcystis aeruginosa). The threshold range for blooms between these taxa was from 10 and 10<sup>2</sup> to 10<sup>4</sup> cells ml<sup>-1</sup>. Often, a major bloom of one taxon would overshadow a less conspicuous bloom of another species (Heterocapsa rotundata) both occurring simultaneously, and responding to favorable growth conditions for their bloom development. Several bloom producing dinoflagellates in this category were also background, or companion species to the more visual blooming taxa, resulting in multiple bloom status for several species at the same time.

Throughout the study period, sporadic bloomers were represented by a diverse assemblage of algae (43). Among these are the 28 bloom producers listed in Table 1. They include 13 dinoflagellates, 7 diatoms, 3 cyanobacteria, 2 euglenophytes, 1 chlorophyte, 1 cryptophyte, and one ciliate (Table 1), with the other species occurring less frequently during this period. Bloom events of record included only those occurring during routine sampling periods, or following special bloom notification and sampling by VDEQ and VDHDSS. Due to daily or seasonal variability in species concentrations, infrequent water analysis, or without an observed color signature, there were likely numerous algal blooms in these waters that were not recorded. Although not inclusive of all bloom occurrences, or taxa that produced blooms during this period, the long term records of these events were considered a representative indication of the bloom species and bloom events in these waters. Of these, the dinoflagellates produced 82% of the recorded blooms, followed in frequency by diatoms (6%) and cyanobacteria (5%), with the other taxa each producing ca. 1-2% of the recorded blooms. There was also the seasonal sequence of taxonomic groups that extended over monthly periods and was repeated annually. For example, the increased diatom concentrations of winter and early spring (e.g. Skeletonema costatum, Skeletonema potamos, Cerataulina pelagica) were subsequently followed by a diverse assemblage of dinoflagellates that produced scattered bloom events throughout these tributaries and which continued into summer and autumn (Marshall, 1994; Marshall et al., 2005). Even when these diatoms were the dominant taxa during this winter/spring period, they also exhibited short periods of sporadic increased cell concentrations at various stations. Other diatoms associated with seasonal sporadic blooms included several Chaetoceros spp., Leptocylindrus minimus, Pleurosigma angulatum, and Thalassiosira nordenskioeldii. Their blooms were more prevalent in the lower reaches of these rivers.

The dinoflagellate *Heterocapsa rotundata* was a common component of the algal flora and a sporadic bloom producer throughout the year, with a bloom threshold beginning at 10<sup>2</sup> cells mL<sup>-1</sup>. Other dinoflagellates having a more dominant presence

#### PHYTOPLANKTON BLOOMS

TABLE 1. Representative bloom producers in Virginia tributaries 1998-2008. \* species more broadly distributed with seasonal bloom development; \*\*Dominant diatoms during spring diatom bloom; @ species considered harmful or toxin producers. Others composition: ¹Chlorophyte, ²Cryptophyte, ³Euglenophyte, ¹Ciliate.

#### Dinoflagellates

Akashiwo sanguinea (Hiraska) Hanse \*@

Alexandrium monilatum (Howell) Balech @

Cochlodinium polykrikoides Margelef \*@

Gymnodinium spp. \*

Gyrodinium spp. \*

Heterocapsa rotundata (Lohmann) Hansen \*

Heterocasa triquetra (Ehrenberg) Stein \*

Karlodinium veneficum (Ballantine) J. Larsen \*@

Pfiesteria piscicida Steidinger et Burkholder @

Pfiesteria shumwayae Glasgow et Burkholder @

Prorocenturm minimum (Pavilard) Schiller \*@

Protoperidinium spp.

Scrippsiella trochoidea (Stein) Loeblich III \*

#### Cyanobacteria

Merismopedia tenuissima Lemmermann \*

Microcystis aeruginosa Kützing \*@

Microcvstis incerta Lemmermann

#### Diatoms

Cerataulina pelagica (Cleve) Hendey \*\*

Chaetoceros spp.

Leptocylindrus minimus Gran

Pleurosigma angulatum (Quekett) W. Smith

Skeletonema costatum (Greville) P.T.Cleve \*\*

Skeletonema potamos (Weber) Hasle \*\*

Thalassiosira nordenskioeldii P.T. Cleve

#### Others

Chlamydomonas spp.1

Cryptomonas erosa Ehrenberg<sup>2</sup>

Euglena spp.3

Eutreptia lanowii Steuer 3

Myrionecta rubra (Lohmann) Jankowski 4

from late spring into autumn included the cyst producers *Heterocapsa triquetra* and *Scrippsiella trochoidea*, plus *Akashiwo sanquinea*. Bloom threshold levels associated with *H. triquetra* and *S. trochoidea* began at 10<sup>3</sup> cells mL<sup>-1</sup>, and for the larger *A. sanquinea* 10 cells mL<sup>-1</sup>. The dinoflagellate blooms were also more prominent in the

lower reaches of these tributaries, whereas, the less saline regions contained increased summer/fall concentrations of cyanobacteria (Microcystis spp., Merismopedia tenuissima) and chlorophytes, e.g. Chlamydomonas sp. (Marshall and Burchardt, 1998, 2004a). Common components throughout these tidal regions were cryptophytes and a diverse assemblage of diatoms. The autotrophic picoplankton produced their greatest concentrations during summer, with diatoms gaining more prominence in late autumn and into winter (Marshall, 1995a; Marshall et al., 2005). Several of the dinoflagellate categories were composed of multiple species under a genus category (Gymnodinium spp., Gyrodinium spp., Protoperidinium spp.), with many of these taxa having sporadic seasonal occurrence with bloom thresholds of ca. 10<sup>2</sup> to 10<sup>3</sup> cells mL<sup>-1</sup> depending on the particular taxon. There also existed dynamic tidal conditions between these rivers, the Chesapeake Bay, and the adjoining Atlantic coastal waters. movements provided access of bloom producing species from these locations to the lower reaches of these rivers and at times produced blooms. These taxa included Eutreptia lanowii, Noctiluca scintillans, Prorocentrum micans, and Protoperidinium spp. Other occasional bloomers entering from the Bay were Ceratium furca and Polykrikos kofoidii.

Among the bloom producing dinoflagellates several taxa have gained additional concern due to being potentially harmful, including Cochlodinium polykrikoides. This species was one of the more prolific and common bloom producer during the warm summer months in several lower Chesapeake Bay tributaries. It has been described by Mackiernan (1968), Zubkoff and Warinner (1975), and Zubkoff et al. (1979) as a reoccurring bloom producer in the lower York River, and is considered potentially toxic and associated with fish kills (Steidinger, 1993). In September 1992, C. polykrikoides produced a bloom that extended southward from the Rappahannock and York rivers that entered many of the tributaries and inlets along the western border of lower Chesapeake Bay. During this period the bloom spread over ca. 215 km<sup>2</sup> of the Bay's central and western regions, then continued beyond the Chesapeake Bay entrance, and progressed to the North Carolina coastal region (Marshall, 1995b). As a cyst producer, the species was able to "seed" various tributaries during this and other bloom events along the southwest shoreline of the Bay to subsequently produce reoccurring blooms in these waters (Seaborn and Marshall, 2008). Thus, C. polykrikoides has established itself in the Lafayette, Elizabeth, and James rivers with annual bloom concentrations appearing in mid-summer and often lasting into autumn. Early stages of the C. polykrikoides blooms generally began at ca. 10<sup>2</sup> cells ml<sup>-1</sup> then soon escalated rapidly in abundance (e.g. >10<sup>3</sup> cells ml<sup>-1</sup>) along with producing a reddish/brown color to the water. An especially long-lasting bloom occurred during August/September 2007 within the lower James River complex, with the bloom lasting 5 weeks at concentrations between 10<sup>2</sup> to >10<sup>4</sup> cells ml<sup>-1</sup>. Detailed discussion of this bloom entering Chesapeake Bay and related water quality relationships have been discussed by Mulholland et al. (2009). Another bloom of this species occurred August 29, 2008 in Knitting Mill Creek, a small tributary of the Lafayette River (Norfolk, VA) with the wind blown surface concentrations along the stream bank at 11.5 X 10<sup>4</sup> cells ml<sup>-1</sup> in addition to a small fish kill. For the past decade this Creek and the Lafayette River have been major bloom sites for this species. These blooms were also associated with high concentrations of cryptomonads in addition to bloom levels of other dinoflagellates (e.g. S. trochoidea, H. rotundata, and Gymnodinium spp.).

Karlodinium veneficum (Gyrodinium galatheanum) has produced blooms in Virginia and Maryland tidal waters from spring to early autumn (Li, et al., 2000, Goshorn, et al., 2004). The toxicity of K. veneficum and its association with fish kills in both agricultural ponds and Chesapeake Bay estuaries have also been reported (Li et al., 2000; Deeds et al., 2002; Goshorn et al., 2004). A major K. veneficum bloom developed in the Potomac River and Virginia inlets to the Potomac that lasted from June through August 2007 at concentrations of 10-33.7 X 10<sup>4</sup> cells ml<sup>-1</sup>. Bloom levels associated with this taxon would begin at ca. 10<sup>3</sup> cells ml<sup>-1</sup>. To date its major blooms regionally occurred in the Potomac River and its associated tributaries. The environmental conditions during blooms of this taxon also supported increased concentrations of other dinoflagellates including A. sanguinea and H. rotundata, among others.

Prorocentrum minimum has been recognized as a major constituent of the flora throughout the Chesapeake Bay estuarine system, and is a common species from early spring into late autumn, with its lowest representation during winter (Tango et al., 2005; Marshall et al., 2006). This was one of the most frequent bloom producers in Virginia tributaries, with bloom thresholds at 10<sup>3</sup> cells ml<sup>1</sup>. Blooms were associated with a reddish/brown coloration to the water and these have been referred to as mahogany or red tides (Tango et al., 2005). These were more common in the higher saline regions of these rivers and less abundant at upstream tidal stations. This taxon is considered a potential toxin producer (Steidinger, 1993; Heil et al., 2005). Brownlee et al. (2005) describe its living resource impact as reducing oxygen concentrations to anoxic and hypoxic levels with Gallegos and Bergstrom (2005) emphasizing these blooms may reduce light availability to submerged plants. concentrations were highest during April to June at 10<sup>2</sup> cells ml<sup>-1</sup>. Records these past two decades have indicated years (1998, 2000, 2003, and 2006) of higher bloom concentrations (10<sup>4</sup> cells ml<sup>-1</sup>), with several sporadic blooms reaching 10<sup>-5</sup> cells ml<sup>-1</sup> in 2000. Blooms of this species have occurred most frequently in Virginia tributaries at temperatures 18-28 °C, salinities of 8-14, and Secchi depth readings < 1.0 m, but it has also been recorded over a wider range of salinities and temperatures. Threshold levels for blooms began at 10<sup>3</sup> cells ml<sup>-1</sup>. Tango et al. (2005) placed this threshold at 3 x 10<sup>3</sup> cell ml<sup>-1</sup>.

Although cyanobacteria are typically associated with freshwater habitats, representative taxa are common within the tidal fresh regions of these rivers, with lower concentrations in the downstream regions of increasing salinity (Marshall and Burchardt, 1998, 2003). Several of these taxa have been associated with toxin production and extended bloom development (Tango et al., 2005; Tango and Butler, 2008). The species of most recent concern has been *Microcystis aeruginosa*. Its mean monthly concentrations in these rivers were ca. 10³ cells ml⁻¹, with lowest abundance levels during winter and highest in summer and autumn. *Microcystis* has produced reoccurring annual blooms in the upper regions of the Potomac River and the adjacent Maryland and Virginia tributaries and inlets along its shoreline and on occasion was associated with high levels of microcystin and health alerts (Goshorn et al., 2004; Tango and Butler, 2008; Marshall et al., 2008a). The blooms were often during periods of rising water temperatures and increased phytoplankton residency time within rivers during summer into early autumn. Threshold status for blooms began at 10⁴ cells ml⁻¹, with health alerts generally at concentrations greater than 10⁴ cells ml⁻¹. Tango and

Butler (2008) reported a July 2003 toxic bloom of M. aeruginosa with concentrations of  $1.6 \times 10^7$  cells ml<sup>1</sup> in a Maryland estuary. To date, similar extensive and long lasting blooms have not been recorded for the Rappahannock, James, York, or Pamunkey tidal regions. Other cyanobacteria associated with blooms in the tidal fresh regions of these rivers have included Microcystis inserta and Merismopedia tenuissima. Other typical fresh water taxa associated with less frequent bloom development include Euglena spp. and Chlamydomonas spp.

Blooms also occurred in these rivers by taxa from a variety of plankton species not typically present in these waters. For instance, the diatom Pseudo-nitzschia cuspidata produced a bloom in the bottom downstream waters of the Potomac River that persisted for several weeks in January 1999. Also, Dinophysis acuminata is a common Atlantic coastal dinoflagellate and potential producer of okadaic acid, the toxin resulting in diarrhetic shellfish poisoning (Marcaillou et al., 2005). When present in the lower Chesapeake Bay D. acuminata concentrations are usually low, with bloom recognition beginning at 10 cells ml<sup>1</sup>. However, it had an extensive bloom in several Potomac River (Virginia) embayments from February to April 2002, reaching 236 cells ml<sup>-1</sup>, with trace amounts of okadaic acid detected at Potomac River locations. Marshall et al. (2003) suggested this species was transported in sub-pycnocline waters northward in Chesapeake Bay to subsequently bloom in these tidal estuaries. Its presence was noted in sub-pycnocline waters in the lower Chesapeake Bay months prior to this bloom. Tyler and Seliger (1978) have previously identified this pathway for the repopulation of *Prorocentrum minimum* into the northern regions of Chesapeake Bay. This sub-pycnocline route may likely represent a conduit for other potentially harmful species to be conveyed from the Atlantic coastal waters into Chesapeake Bay regions and its sub-estuaries. Other species that may have followed a similar path of entry would include P. cuspidata mentioned above and the dinoflagellate Noctiluca scintillins, which is common to neritic waters, and has produced blooms in the lower James River (1987, 2000) and Chesapeake Bay (2002) (Marshall, 1995b).

Blooms of the ciliate Myrionecta rubra (Mesodinium rubrum) containing the redpigmented cryptophyte endosymbiont have occurred frequently in Chesapeake Bay and in the lower regions of the Potomac, Rappahannock, York, and James rivers. In October 1995 a major bloom of M. rubra developed in the lower Chesapeake Bay with concentrations of ca. 500 cells ml<sup>-1</sup> (Marshall, 1996). Another more recently reported taxon in Virginia waters is the dinoflagellate Alexandrium monilatum. It was first identified during routine sampling in September 2007 at sites in the York River at bloom concentrations of ca. 1,200 cells ml<sup>-1</sup> (Marshall et al., 2008b). This is an ichthyotoxic species and commonly produces cysts following bloom development (Walker and Steidinger, 1979). There was a September 2008 and 2009 re-occurrence of this taxon within the York River, and in September 2009 also in the lower Chesapeake Bay at concentrations 125-256 cells ml<sup>-1</sup>. These sequential yearly records imply that this species has established itself in this region (possibly enhanced through cyst development) and has now become an annual bloomer with the potential of spreading its range into other tributaries of Chesapeake Bay.

#### Discussion

Phytoplankton blooms were common events within Virginia's tidal tributaries. They occurred frequently and were produced by a variety of species. These results support those of Parker (1987) and Smayda (1997) in that what characterizes a bloom is species specific and is directly influenced by cell size, pigment content, and cell abundance. Each taxon will respond to those environmental conditions favorable to its continued development, which frequently results in bloom concentrations, and a visible color signature in the water. The bloom threshold concentrations given here provide standards recommended for identifying bloom status among various algae in these tidal rivers.

Depending on the taxa, the threshold range for an algal bloom in these waters varied from 10 cells m $l^{-1}$  to  $>10^4$  cells m $l^{-1}$ . Although many of the blooms developed annually and became common occurrences, there were others that reached bloom status infrequently or represented latent populations of earlier recorded bloom producers. Pfiesteria piscicida and P. shumwayae were associated with blooms and fish kill events in Maryland tributaries in 1997. Detailed specifics regarding their occurrence and toxicity have been reported by Glibert et al. (2001), Duncan et al. (2005), Gordon and Dyer (2005), and Moeller et al. (2007). Glibert et al. (2001) also reported the 1997 blooms of P. piscicida in Maryland were not repeated in 1998, but were replaced by huge P. minimum blooms. Our present monitoring of Pfiesteria spp. by molecular genetic analysis indicated only a sparse and scattered presence of these taxa (mostly P. shumwayae) in Virginia tributaries, with no bloom events associated with these taxa in recent years. However, these species have remained present in these tributaries and still may respond to environmental conditions favorable to bloom development. The re-occurring bloom development of other taxa remained sporadic and unpredictable (e.g., D. acuminata, N. scintillins), with other indigenous species representing a category of consistent bloom producers (including H. triquetra, P. minimum, S. potamos, S. costatum).

Marshall (1989) reviewed reports of blooms occurring 1960-1989 within the Chesapeake Bay estuarine complex and noted a greater occurrence of blooms in the creeks and rivers entering the Bay (67%), with their highest incidence (54%) taking place during summer. Bloom concentrations were generally identified with taxa having 10<sup>3</sup> to 10<sup>4</sup> cells ml<sup>-1</sup>. Major bloom producers during this earlier period included P. minimum, H. triquetra and H. rotundata. The present results agree that these same taxa are common bloom producers with high abundance in the regional rivers and streams. Presently >1,400 phytoplankton species have been identified within the Chesapeake Bay estuary system, with 38 (2.5%) recognized as potentially harmful species (Marshall et al., 2005, 2008a). This study identified 28 species associated with the more common sporadic blooms, including 8 considered potentially toxic or harmful species. These were the cyanobacterium M. aeruginosa, and an assemblage of dinoflagellates respresented by A. sanguinea, A. monilatum, C. polykrikoides, K. veneficum, P. piscicida, P. shumwayae, and P. minimum. Although these species represented a fairly small component for these waters, they were a potential source of serious environmental consequences (e.g. fish kills, shellfish contamination, and human illness), with other potentially harmful taxa likely to enter and populate these waters in the future.

Blooms were seasonally produced by a resident population of indigenous taxa, plus the occasional appearance of transient species and their subsequent bloom development. In general, favorable conditions for algal growth and bloom development existed in these rivers. A variety of these blooms were associated with rising water temperatures, increased phytoplankton residency time within these rivers, and an adequate nutrient supply. These conditions provided time for expanded algal bloom development and increased opportunities for bloom taxa to enter adjacent waters and continue to reintroduce cells to the rivers and maintain bloom status.

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Francis Burke Leftwich

Dr. Francis Burke Leftwich, Fellow and former president of the Academy, passed away at the age of 76, on Wednesday, February 10, 2010, with complications of lymphoma. He was born October 4, 1933 in Glen Allen, Virginia. He was the youngest of five brothers, one sister, and the son of late Charles Beverly Leftwich and Lucille Gallion Leftwich. Dr. Leftwich graduated with a B.A. from the University of Richmond in 1956, and received a Masters of Science from U of R in 1958. He received a Doctor of Philosophy from the University of Tennessee, Knoxville in 1963. Following a postdoctoral fellowship at Rutgers University, he returned to the University of Richmond where he taught Biology and Endocrinology from 1964 to his retirement in 1996. From 1985 to 1996, Dr. Leftwich served as the Chair of the Biology Department at the University of Richmond during which time he oversaw the design and construction of the original Gottwald Science Center in 1978. At the re-dedication of the Science Center in 2006, a pre-med counseling center was named in his honor. In 1976, Dr. Leftwich received the University of Richmond's distinguished educator award. He counseled pre-med students and worked with graduate students on research projects ranging from how frogs change color to the function of the pineal gland in rats. He was an avid fisherman and loved gardening, especially roses and camellias. As long and faithful supporter of the Academy, he encouraged his students to present at our meetings and become members. He was elected president and Fellow of the Virginia Academy of Science in 1984.

Survivors include his wife of 55 years, Frances Stallard Leftwich; daughters, Dr. Julie Beales of Henrico County, Amy Moore and Sarah Branch, both of Henrico, and Kathryn Muir of Charlotte, N.C.; a sister, Caroline Hodgskin of Orlando, Fla.; and 12 grandchildren.



### FRANKLIN D. KIZER

Academy Fellow, Franklin Dadmun Kizer, 93, of Lively, Virginia, died on March 13, 2010. Surviving are his wife of 72 years, Helen B. Kizer; daughters and sons-inlaw, Ann K. and Melvin G. Spain, of Mechanicsville, and Marion K. and Merlin M. Renne of Williamsburg; six grandchildren and seven great-grandchildren. He was son of the late Franklin J. Kizer and Marion B. Kizer of Norfolk. He received his bachelor and master of arts degrees from East Carolina University in 1942 and 1949. After college, he was a chemist and ship safety inspector at Norfolk Naval Shipyard. Thereafter, he taught chemistry and physics in Norfolk from 1949 until his appointment in 1956 as the first State Supervisor of Science for the Virginia Department of Education in Richmond. He served in that capacity with great distinction until his retirement in 1979. Mr. Kizer was a co-founder and first president of the Council of State Science Supervisors in 1963. Thereafter, he served as its Executive Secretary for 25 years, during which he directed several of its national conferences as well as seven regional conferences for the National Science Foundation. He joined the Virginia Section of the American Chemical Society in 1954 and served as its Chairman throughout 1976. Mr. Kizer is the only individual to have received both its Distinguished Service Awards for high school chemistry teaching and for contributions to the chemical profession.

Published in Richmond Times-Dispatch on March 15, 2010

## **NOTES**

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# A Standardized RNA Isolation Protocol for Yam (Dioscorea alata L) cDNA Library Construction

Satya S. Narina, Virginia State University, Petersburg, Virginia;
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Robert Asiedu, International Institute of Tropical Agriculture (IITA),
Nigeria; and H. D. Mignouna, African Agricultural Technology
Foundation (AATF) c/o ILRI, P.O. Box 30709, Nairobi, Kenya

#### **ABSTRACT**

For the purpose of constructing yam cDNA libraries, attempts to isolate high quality RNA using several previously reported protocols were unsuccessful. Therefore a protocol was standardized for yam total RNA isolation by using guanidium buffer at the Department of Biology, Virginia Sate University. The RNA isolated using this standardized protocol was high in quality and led to successful good quality cDNA library construction and identification of functional ESTs in yam.

#### INTRODUCTION

Yam, (Dioscorea alata L), is the main food source for over 100 million people in humid and sub-humid tropics. Its production is affected by several biotic and abiotic factors (Abang et al., 2003). Anthracnose, caused by Colletotrichum gloeosporioides, is the most severe foliar disease of water yam (Dioscorea alata L) and is a major hurdle in yam production. It is reported that anthracnose causes yield reduction up to 90% (http://annualreport.iita.org). There are no cost effective control measures and the long-term solution to the problem will be through the development of resistant genotypes (Mignouna et al., 2002. Very limited yam sequence information is available from public genome databases. A review of previous efforts to develop cDNAs towards EST development in yams revealed that housekeeping genes were prevalent in the libraries constructed using total RNA from male flowers (Mignouna et al., 2002a, b, c).

It is realized that obtaining high quality, intact RNA is the first and the most critical step in conducting cDNA library construction and for further analysis of gene of interest. After many attempts of total RNA isolations from yam leaf samples using standard plant RNA isolation protocols (Verwoerd et al,1989), only 6-10 ug of total RNA was extracted from the leaves and no colonies were observed when this RNA was used for cDNA library construction. The RNA appeared as a smear on 1.1% agarose gel (Fig. 1). The most likely reason for not getting good quality RNA is the mucilagenous tissue in yam plant parts like leaf, stem and tuber. This tissue causes problem because of polyphenols, polysaccharides and other secondary metabolites that are rich in yam plant parts and are not easily removed by conventional extraction methods. The aim of this study was to establish a protocol for RNA isolation from Dioscorea alata to get high quality and high quantity RNA that is suitable for generation of molecular markers, such as EST-SSRs and SNPs. Therefore, the following article discusses successful and reproducible method of RNA isolation

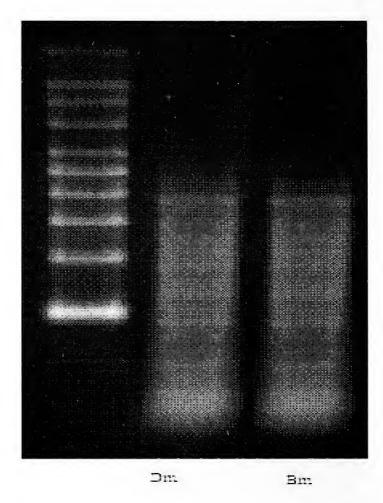


FIGURE 1. A smear of rRNA samples of Dm-Resistant yam genotype and Bm-Susceptible yam genotype isolated using standard protocols on 1.1% Formaldehyde agarose gel

procedure employed for yam cDNA library constrcution and ways of increasing RNA yields

#### MATERIALS AND METHODS

Tissue collection: In order to standardize the protocol for RNA isolation, the yam (source: local grocery store) were grown in the green house in pots. Fresh 1g leaf tissues are collected in 50ml BD Falcon tubes, frozen quickly in liquid nitrogen.

RNA isolation. Only the successful procedure of RNA isolation with the modifications to standard plant RNA isolation protocol is reported here.

#### Solutions and solvents used:

- Extraction buffer (100 ml stock): 76.424g of 8M Guanidium Hydrochloride + 425 mg of 20mM MES + 740mg of 20mMEDTA+ 35ml of DEPC water. Adjust the pHwith 10M NaOH, autoclave and store at 4°C. Add 1.38µl of β-mercaptoethanol (50mM) just before use.
- Phenol: Cholorform: Isoamulalcohol (24:23:1)

#### Procedure:

- 1. 1g tissue ground in liquid nitrogen was homogenized in 2ml extraction buffer + 2ml Ph:Chl:IAA. {The sample was homogenized using power operated mini grinder (the steel grinder part was pre-cooled in liquid nitrogen) that perfectly fits in to the falcon tube. It was necessary to maintain frozen conditions throughout the extraction to enhance the quality of the target RNA. }.
- 2. The sample was centrifuged for 10 min at 10,000rpm (at 0-2 °C).
- 3. To the Supernatant, Ph:Chl:IAA (equal volumes in 1:1 ratio) was added and the RNA was precipitated overnight in -20.
- 4. The next day the sample was centrifuged for 20 minutes at 10,000rpm (at 0-2 °C) and the pellet and was dissolved in Deionized water (Volume based on required concentration).
- 5. RNA was stored at -80°C. The quality of RNA was confirmed by using BIO-RAD Smartspec<sup>™</sup> plus Spectrophotmeter and also by Formaldhyde agarose gel electrophoresis (Sambrook et al, 1989).

#### cDNA LIBRARY CONSTRUCTION

The freeze dried leaves of D. alata L genotypes, Tda 95/00328, resistant to the FGS strain of C. gloeosporioides but susceptible to the SGG strain and TDa 92-2, susceptible to the FGS and SGG strains of C. gloeosporioides were obtained from IITA, Ibadon, Nigeria. Leaves were ground in liquid nitrogen and total RNA was isolated using the standardized protocol. Total RNA thus isolated was used for the construction of cDNA library using The Creator smart cDNA library construction kit (BD Biosciences Clonetech). First strand cDNA was synthesized using SMART IV oligonucleotide followed by long distance PCR amplification to generate high yields of full-length ds cDNAs (~400 to >4000 bp) followed by Sfi I digestion and column fractionation. The cDNA fractions that match the desired size distribution (1-4kb) were selected. The Sfi I - digested cDNA was ligated to the Sfi I digested dephosphorylated pDNR-LIB Vector (Clonetech) and transformed into DH10B T1 Phase resistant bacterial cells. The chloramphenical resistant colonies were picked and archived in 96 well plates. For preliminary round of sequencing, about 100 colonies from each library (resistant and susceptible) were randomly selected and subjected to single pass sequencing (Agencourt Biosciences).

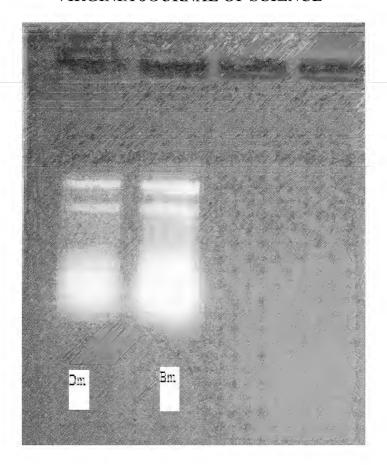


FIGURE 2. Intact yam rRNA samples using current protocol.

#### RESULTS AND DISCUSSION

The quantity of total RNA is between 250 to  $500\mu g$  from 1g of yam leaf tissue. The 18S and 28S ribosomal RNA bands are clearly visible in the intact leaf RNA samples Dm and Bm of yam (Fig. 2) and the quality reading on spectrophotometer were presented in the Table 1.

Following quality check of the sequences, the pure quality sequences were checked for homology to sequences in GenBank using BLAST similarity search tool. Data obtained from the BLAST analysis of 100 clones from each resistant (Dm) and susceptible (Bm) accessions were compiled and interpreted with respect to the hits identified in other plant species (Table 2 and 3).

This preliminary data describes the initial efforts to develop tools to annotate EST's for anthracnose disease resistance genes by constructing good quality cDNA libraries for different accessions of *D.alata*. From each cDNA library 6000 colonies were arrayed into 96 well plates. A total of 100 clones randomly selected each from two

Sample	ng/μL	A260	A280	A260	A260	Constant	Cursor	Cursor	340
ID				A280	A230		Pos	Abs	raw
Bm	257.6	6.438	2.997	2.15	1.5	40	230	4.287	0.082
Dm	309.6	7.741	3.646	2.12	1.15	40	230	6.703	0.022
									1

TABLE 1. Spectrophotometer readings of quality RNA samples from yam genotypes.

distinct libraries namely Dm and Bm. Of the 100 cDNA clones from each yam genotype, 10 yielded no sequence and an additional 9 produced sequences of less than 100 bp and these were not used for sequence analysis. The average length of the remaining sequences was 762 bp.

Based on top Blast hits in plants, in yam type Bm, out of 100 sequences, 48 were distinct gave >400bp and were showing functional similarities. In Yam type Dm, out of 100 sequenced clones 48 were distinct, gave >400bp and 22 were duplicates of yam type 1 were observed. The genes putatively identified are shown in Table 2 and 3. The blast hits identified in different crops showed 88-100% identity and, in general, the homology of the insert sequence to the blast hit is about 400-500bp out of 700-800 bp length aligned. The genes (ESTs) identified based on sequence similarity are involved in various putative functions such as gene or protein expression, protein binding, ripening, cell wall and stress response, defense, photosynthesis, photoperiodic flowering response, cell division and proliferation, nodulation, and secondary metabolism etc. and some of them could not be classified into any of these categories.

The numbers of hits showing stress/defense related function were comparatively more in resistant genotype when compared to susceptible genotype (Satya et al, 2007). Of the distinct sequences there are sequences similar to unknown protein and unknown mRNA (1-2%) not presented here. The information on hits to clone sequences (10%) in different crop species and the top blast hits to mitochondrial genes and genes encoding for ribosomal protein genes (20%) were not listed in the table. By sequencing a large number of cDNAs, we can selectively avoid the clones that represent ribosomal and mitochondrial genes, and choose clones that represent genes that we wish to examine. This is a significant improvement compared to previous efforts where sequences coding for ribosomal proteins were predominant in the libraries. This achievement is attributed to quality RNA isolation.

#### CONCLUSION

Two cDNA libraries for yam, one each for resistant and susceptible genotypes, were constructed for the purpose of identifying clones that are differentially expressed in these two genotypes. Many new genes have been identified that can be useful for future studies. The sequences may also be a source of single-nucleotide polymorphisms or simple sequence repeats for molecular marker development.

Preliminary analysis of 200 clones revealed homologies to known genes in several related and distant plant species. Though the numbers of hits were comparatively more in resistant genotype compared to susceptible genotype, not much distinct differences were observed between the functional hits to sequences of these two genotypes.

TABLE 2. Blast hits from cDNA library of Yam accession Dm

Clone ID	NCBI Definition line for	Crops in which hits were identified
	Putative function of the	
	Blast hits	
Dm 3	mRNA, complete cds;	AC183495.1(Cabbage);
		gb DQ903665.1 (Turnip);
		dbj AP008209.1 (Rice)
Dm 4	genomic DNA, chromosome	dbj AP008207.1 ; dbj AP001633.2 ,
	1,10	dbj AP008209.1(Rice)
Dm 7, 52	Metallothionein-like protein	DQ202305.1(sago palm);
	(MET,grip24,MWMT3)	AJ236913.1(African oil palm);
	mRNA, complete	AJ237990.1(grape);
\		AY857933.1(Cotton);
_		AF268393.1(Banana)
Dm 8	Solanum lycopersicum	dbj AP009293.1  (brinjal);
	genomic DNA, chromosome	NM_001073500.1,
	8, clone: C08HBa0323O07,	AP008218.1(Rice)
	complete sequence;Oryza	
	sativa (japonica cultivar-	
	group) genomic DNA,	
- 11	chromosome 12	
Dm 11,	chloroplast, complete	DQ887676.1(Drimys)
32, 69, 59	genome	AJ627251.1(Nymphaea alba);
		AY916449.1(Phalaenopsis
		aphrodite); DQ899947.1 (tulip)
Dm 12, 45	chloroplast mRNA for Tic62	AY437888.1(Sheperd's purse);
	protein;IbJ8 mRNA for JA-	AJ344551.2(Pea);
	domain, complete cds;	DQ499754.2(Potato);
	SrGLU5 mRNA for beta-1,3-	AB246796.1(Sweet potato);
	glucanase, complete cds	AB242267.2(Sesbania);
		AB210846.1(Lemna)
Dm 15	Ribulose-bisphosphate	NM_123204(Arabidopsis).3;
	carboxylase (AT5G38430)	V00458.1(Soybean); AY143814.1;
	mRNA, complete cds;	AY142543.1; AY065026.1
Dm 18	aci-reductone dioxygenase-	DQ244304.1; AY103746.1(Maize);
	like protein (ARD) mRNA,	CT831853.1, NM 001055581.1,
	complete cds	AY955841.1(Rice);
	r	AB025597.1(Barley)
Dm 21	Oryza sativa (japonica	dbj AP008208.1
2111 21	cultivar-group) genomic	aojiri oobooni
Dm 24	DNA, chromosome 2	NIM 001022522 1
	mRNA sequence complete	NM_001032532.1
	cds	

Dm 30	Oryza sativa microsatellite	AY023257.1
	MRG5582 containing	
	(GGA)X13, genomic	
Dm 34	Medicago truncatula clone	AC136839.20
	mth2-13n2, complete	
	sequence	
Dm 42	Full-length cDNA Complete	CR936947.2 , emb
	sequence from clone	CR931731.1 (Medicago);
		BX821860.1(Arabidopsis)
Dm 48	Glycine max mRNA for asparagine	gi 109940718 emb AM158274.1
Dm 53	cDNA clone:full insert,	NM_001066955.1;
	mRNA sequence, complete	AK070897.1(Rice);
		AY107920.1(Maize)
Dm 54	Zea mays cultivar Mo17	AY664418.1
	locus 9008, complete	
Dm 58	sequence	A COOCETT 2: A DOTTI 20 1
Dm 36	Sequence of BAC F15I1 from Arabidopsis thaliana	AC006577.2; AB077139.1
	chromosome 1; Prunus	
	persica DNA, microsatellite	
	marker MA035a	
Dm 59	Croomia pauciflora large	DQ629350.1(Nuttall);
	subunit ribosomal RNA gene,	DQ629349.1(Yam)
	partial; Dioscorea sp. Qiu	
	94044 large subunit	
Dm 61,	ribosomal RNA gene, partial  Drosophila melanogaster	AE014296.4(Drosophila);
Dm 80	_	
DIII 00	chromosome 3L, complete	AJ831399.1(Santalum)
	sequence; Santalum	
	austrocaledonicum	
	microsatellite DNA, clone	
D (4	mSaCIRF04	A.E.4.1.004.1
Dm 64	Lycopersicon esculentum	AF411804.1
	BAC clone Clemson_ID	
D (6	47I13, complete	PROSICAL CALCO
Dm 65	histidine ammonia-lyase-like	EF051316.1(Gymnadenia);
	mRNA, complete	BT012683.1(Tomato);
		AC157536.31(Medicago)
Dm 70	Oryza sativa (japonica	AP008209.1
	cultivar-group) genomic	
	DNA, chromosome 3	
Dm 79	Zea mays clone 92533	DQ245928.1
	mRNA sequence	
Dm 88	Brassica rapa subsp.	AC189444.1
	pekinensis clone	
	KBrB070J23, complete	

Dm 89	Sorghum bicolor clone SB_BBc0020O07, complete sequence	AC169375.4
Dm 91	Arabidopsis thaliana unknown protein (AT2G19830) mRNA, complete; Lycopersicon esculentum clone 134156F, mRNA sequence; Zea mays clone EK07D2310A10. c mRNA sequence	NM_127541.2; BT013152.1(Tomato); BT017005.1(Maize)

TABLE 3. Blast hits from cDNA library of Yam accession Bm

NCBI Definition line for Putative function of the	Crops in which hits were identified
Blast hits	
Metallothionein-like protein	DQ202305.1(Sago palm),
(MET) mRNA, complete	AJ236913.1(African oil palm),
	Grape, cotton, citrus, musa and rice
Mitochondrial, chloroplast	DQ887676.1(Drimys granadensis);
DNA, complete sequence;	AJ627251.1(Lotus);
ribosomal RNA gene partial	AB240139.1(Tobacco);
	DQ629360.1(Dicentra Sp.);
	DQ340440.1(Pacific Dogwood),
	DQ923117.1(Heavenly Bamboo);
	AF205123.1; DQ629349.1,
	DQ629457.1(yam);
	DQ629350.1(Nuttall)
	CT829335.1(Rice);
clone:OSIGCFA011A01, full	AY224463.1(Rice)
insert sequence;	
mRNA for Mob1-like protein	AY437888.1(shepherd's purse),
(mob1-B) complete cds	AM161645.1(alfalfa)
LpLHY H2 mRNA for LHY	AB210846.1(Duckweed),
homologue2, complete	DQ499754.2(Potato)
unknown protein	NM_130173.3(Arabidopsis),
(AT2G46100) mRNA, complete	BT012819.1(tomato)
	Putative function of the Blast hits  Metallothionein-like protein (MET) mRNA, complete  Mitochondrial, chloroplast DNA, complete sequence; ribosomal RNA gene partial  cDNA clone:OSIGCFA011A01, full insert sequence; mRNA for Mob1-like protein (mob1-B) complete cds  LpLHY H2 mRNA for LHY homologue2, complete unknown protein (AT2G46100) mRNA,

Bm 35,	lipid transfer protein mRNA,	EF031153.1(Stevia);
63	complete cds	AY395741.1(Summer grape)
Bm 43	beta-1,3-glucanase,	DQ499754.2(Potato);
	complete,cds	AB246796.1(Sweetpotato);
		AB242267.2(Sesbania)
Bm 47	Oryza sativa (japonica	AP008207.1(Rice)
	cultivar-group) genomic	
	DNA, chromosome 1	
Bm 48	aspartic proteinase 4,	CT829760.1(Rice);
	complete cds	AB045894.1(Nepenthes);
		NM_001049320.1(Rice);
		AY103982.1(Rice)
Bm 55	putative terpene synthase,	AK227599.1(Arabidopsis);
	complete cds	NM 001054333.1(Rice)
Bm 59	Full-length cDNA Complete	BX817199.1(Arabidopsis)
	sequence from clone	
Bm 60	Brassica rapa subsp.	AC189190.1(Chinese cabbage);
	pekinensis clone	DQ144500.1(sheperd's purse)
	KBrB002G19, complete;	
	Capsella bursa-pastoris	
	ecotype CZ96 microsatellite	
	ATCP70189	
Bm 32,	cDNA clone: full insert	AC137065.26(alfalfa),
61, 62,	sequence;mRNA, complete	DQ244538.1, DQ245784.1,
64	sequence	DQ244442.1(maize);
		CT830019.1(Rice); AK069033.1,
		CT829171.1, CT830462.1(Rice);
		AP006116.1(Lotus);
		BT014284.1(Tomato);
-		AY085715.1(Arabidopsis);
Bm 75	Lotus japonicus genomic	AP004531.1(Lotus)
	DNA, chromosome 3, clone:	
D 17	LjT13M14	A D 22 ( 9 ( 7 1 ( C ; ) )
Bm 17,	Nicotiana tabacum	AB236867.1(Ginseng);
39, 80	chloroplast pigment-binding	DQ676843.1,
	protein CP29 (Lhcb4); Panax	AY219853.1(Tobacco);
	ginseng cab mRNA for	CT829715.1(Rice)
	chlorophyll a/b binding protein; Nicotiana tabacum	
	chlorophyll a/b binding	
	protein mRNA, complete	
Bm 82	ubiquitin conjugating enzyme	L29077.1(Peas);
DIII 02	(UBC4), complete cds	CT833517.1(Rice);
	(OBC4), complete cas	AY086109.1(Arabidopsis)
Bm 92	Populus trichocarpa clone	AC182669.2(Populus)
DIII 74	Pop1-21I14, complete	AC162009.2(ropulus)
	sequence	
Bm 94	Glycine max mRNA for	AM158274.1(Soybean)
	asparagine synthetase, type	
	III (sas3 gene)	

Therefore this project is revised to continue cDNA library construction from challenged leaf tissues of these two genotypes besides including a third genotype resistant to SGG strain to identify the candidate genes to anthracnose resistance. The ESTs generated in this study also provide a good tool for more studies to understand the resistant and susceptible interactions of yam anthracnose.

Analysis of sequences from recently completed revised yam genomics project will generate more ESTs for differential expression analysis for the purpose of identifying candidate genes for anthracnose resistance, marker development and further yam QTL mapping studies.

#### **ACKNOWLEDGMENTS**

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S.Narina.,A.Mohamed.,R.Asiedu and HD Mignouna were involved in the design of the study, carried out the molecular experiments, analysed the data and drafted the paper. R. Asiedu supplied the material and drafted the paper. All authors read and approved the final manuscript.

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## Management and Social Indicators of Soil Carbon Storage in a Residential Ecosystem, Midlothian, VA

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#### ABSTRACT

Soil carbon storage - defined here as carbon mass per unit ground area - is an important ecosystem service, sequestering carbon that might otherwise exist in atmospheric CO<sub>2</sub>. Significant attention has focused on the effects that humans have on carbon cycling, but little is known about how human behaviors and attitudes relate to lawn carbon storage. The objectives of this study were to conduct household surveys in concert with soil carbon sampling in a 10-yr-old exurban neighborhood near Richmond, Virginia to quantify differences in soil carbon storage between residential lawns and mixed pinehardwood forest fragments, and to determine how lawn management and environmental attitudes relate to soil carbon storage. Lawns stored significantly less carbon than forest fragments in the top 10 cm of soils. A significant negative relationship was observed between watering and fertilizer frequency and soil carbon storage, but the goodness-of-fit was sensitive to intra-lawn variability in soil carbon mass. Survey respondents that claimed to be environmentalists stored significantly more carbon and spent one hour less per week managing their lawns, suggesting that environmental attitudes may affect how households manage their lawns and, in turn, the quantity of soil carbon stored in residential soils.

Key words: Soil carbon, carbon sequestration, lawn, human-dominated, residential, management

#### INTRODUCTION

Deforestation during land-use conversion is a principal determinant of the carbon balance of the conterminous United States, prompting C emissions of 12 Tg yr<sup>-1</sup> from 1990-2004 (Woodbury et al. 2007). A substantial fraction of C emissions to the atmosphere in the U.S. is attributed to land-use conversion from forest to residential ecosystems, which increased from 2.5 to 3.1% from 1990-2000 (Nowak et al. 2005). Although disturbance during land-use conversion may cause an initial precipitous decline in soil carbon storage, defined here as soil carbon mass per unit area, post-

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conversion management of residential lawns is hypothesized to prompt recovery of soil carbon storage that in some cases exceeds that of undisturbed forests (Pouyat et al. 2006, Pouyat et al. 2007, Pickett et al. 2008). The proposed mechanism for higher soil carbon in highly productive residential lawn 'ecosystems' is the accumulation of grass derived organic matter (Milesi et al. 2005). Nowhere in the U.S. is land-use conversion from forest to residential habitat more prominent than in the southeastern Piedmont region, which is now experiencing net deforestation for the first time in nearly a century as the region rapidly urbanizes (Polsky et al. 2000).

The amount of soil C stored in residential ecosystems following land-use conversion from forests is determined by the balance of carbon inputs and outputs over time, and carbon storage prior to land-use conversion (Pickett et al. 2008). Humans substantially alter the balance of carbon inputs and outputs through management of residential lawns (Baker et al. 2007). Management regimes that augment lawn net primary production (i.e., growth) and retain grass clippings increase carbon inputs to residential soils (Heckman et al. 2000, Kopp and Guillard 2002, Qian et al. 2003). Effects of common lawn management practices on soil organic matter decomposition and, consequently, carbon outputs via microbial respiration are less certain, but soil disturbance generally causes carbon losses (Kaye et al. 2005).

Despite its probable importance, lawn management at the household scale plays an uncertain role in determining soil carbon storage in residential ecosystems, especially in the understudied, urbanizing Piedmont region of the southeastern U.S. (Kaye et al. 2006, Pataki et al. 2006, Pickett et al. 2008). The Virginia Piedmont is a model location for examining the presently poorly understood consequences of land-use transformation on carbon cycling because urban growth is equal to or greater than that of many areas in the region (Rogers and McCarty 2000). Numerous studies conducted in other geographic regions have quantified carbon storage in soils and other pools within human-dominated ecosystems (Jo and McPherson 1995, Koerner and Klopatek 2002, Nowak and Crane 2002, Kaye et al. 2004, Ziska et al. 2004, Kaye et al. 2005, Milesi et al. 2005, Golubiewski 2006, Groffman et al. 2006, Pouyat et al. 2006). Only a few studies have taken soil carbon analyses a step further by empirically examining soil carbon storage across a range of lawn management intensities (Qian and Follett 2002), or in relation to social indicators that, in human-dominated ecosystems, may be robust integrated predictors of soil carbon properties (Pataki et al. 2006, Pickett et al. 2008). Additionally, educational campaigns that aim to reduce household carbon footprints require knowledge of how residential soil carbon storage relates to human behavior and attitudes. For example, carbon footprint models that predict the carbon signature of households from human behavior are proposed tools for educational outreach and behavior interventions (Dilling et al. 2003, Pataki et al. 2006, Dilling 2007b).

Here, household surveys of lawn management behavior and environmental attitudes were conducted, and soil carbon mass (to 10 cm depth) quantified in lawns and forest fragments of a 10-yr-old exurban neighborhood near Richmond, Virginia to: 1) determine whether soil carbon storage differed between forests and lawns; and 2) identify lawn management, physical, and social indicators of soil carbon mass in a residential ecosystem. An important secondary objective of this work is to examine the feasibility of developing simple models for predicting soil C storage from lawn management practices. The study focuses on an understudied system that is increasingly typical of the Piedmont region of southeastern U.S. in which a planted pine

forest with hardwood representation is converted to a residential ecosystem (Polsky et al. 2000). The Piedmont comprises a distinct physiographic region of the U.S. with unique soils, climate, and socio-demographic features, suggesting that soil carbon patterns may differ from those reported by prior studies conducted in other geographic areas. Additionally, this study is among the first to link human management behavior and attitudes to soil carbon storage following land-use conversion from forest to residential lawn.

#### METHODS

Study location

The study was conducted in an upper-middle class exurban neighborhood located 30 km west of Richmond in Midlothian, VA (zip code 23112). Single family homes >300 m² were built in the middle to late 1990s on residential parcels ranging from 0.25 to 0.5 ha. Human population density was 6440 km² with a median household size and annual income of 3.26 and \$85,000 USD, respectively, in the year 2000 (United States Census Bureau 2000).

The dominant ecosystem prior to land-use conversion to a suburban residential neighborhood, as indicated by the surrounding forest fragments, was a mature forest typical of the region comprised of planted loblolly pine (*Pinus taeda* L.) and volunteer hardwood competition including oak (*Quercus*), hickory (*Carya*), and maple (*Acer*) genera. Homes within the suburban neighborhood are distributed throughout fragmented forest remnants, which are preserved in common space that is used for recreation (Figure 1). Residential soils are a Creedmoor fine sandy loam on slopes of 0-12 % (USDA 2009). Mean January and July air temperatures are 6.1°C and 25.6°C, respectively, with mean annual air temperatures of 14.3°C and mean annual precipitation of 1115 mm (NOAA 2009).

#### Experimental design and household surveys

The experimental design coupled household surveys of lawn management and environmental attitudes with lawn soil bulk density and carbon sampling in a single neighborhood. To examine impacts of forest conversion to lawns, soils were sampled in residential lawns and adjacent forest fragments distributed throughout the neighborhood. The examination of a single, exurban neighborhood minimized variation in soil properties caused by differences in time since land-use conversion, climate, soil type, parcel size, and household affluence, all of which may constrain soil carbon percent and mass (Pickett et al. 2008).

Sixty household surveys of lawn management behavior and environmental attitudes randomly distributed within the study neighborhood in the autumn of 2008 yielded 33 respondents. Surveys inquired about: a) the application, irrespective of frequency, of soil aeration, lawn clipping retention, raking of detritus, mulch, chemical weed control, chemical pesticides, and seeding; and b) the frequency of mowing, watering, and bagged fertilizer application during the autumn, spring, and summer months. Two additional questions asked respondents to describe their alignment with environmental issues and how many hours per week they work in their lawns during the growing season.

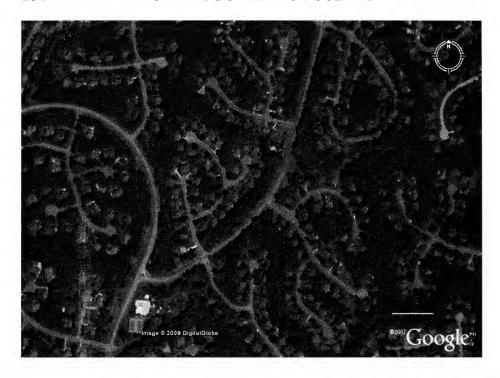


FIGURE 1. Aerial view of the residential neighborhood examined for soil properties in Midlothian, Virginia, 30 km outside of Richmond. The inset ruler is 100 m.

#### Soil bulk density and carbon percent and mass

The O horizon and mineral soil carbon were sampled together to a 10 cm depth during the dormant season of 2008-2009 in 33 residential lawns for which management practices were determined via household surveys, and in 24 randomly selected locations within three large, contiguous forest fragments encompassing the residential areas. Sampling was limited to the top 10 cm since this carbon-rich soil surface pool is likely more sensitive to disturbance, including land-use transformation from forest to lawn, and also is more responsive to subsequent management (Pouyat et al. 2006). To minimize potential bias from recently deposited detritus (e.g., leaf litter, woody debris), fresh litter (O, horizon) was removed from the surface prior to using a metal corer (3.0 cm diameter) to extract soil from each sampling location. It is important to note that because of composite sampling of O and mineral soils, physiochemical properties reported are a combination of both horizons. In each residential lawn, soil subsamples were collected at three locations 10 m apart along a transect running parallel to each house. As a proxy for local microclimate, aspect and slope were recorded for each soil collection location. Forest soils were collected from locations with no visible disturbances that were > 20 m interior to the forest boundary and within the common area of the neighborhood. Soils were stored at -20°C until processed.

In the laboratory, soils were sieved through a 2 mm mesh screen, remaining roots

TABLE 1. Summary of soil properties (to 10 cm depth) and statistics for lawns (n = 33 lawns) and forest fragment soils (n = 24) in Midlothian, VA.

Parameter	Lawn	Forest fragment
Bulk density (g cm <sup>-2</sup> )	0.92 (0.035)*	0.41 (0.037)*
Percent carbon	3.85 (0.27)*	16.54 (1.04)*
Carbon mass (kg m <sup>-2</sup> )	3.30 (1.45)*	5.50 (1.24)*

Mean  $\pm 1$  standard error, \*P < 0.0001

were removed, and root-free soil was dried in an oven at 60°C to a constant mass and weighed to obtain bulk density. A portion (~ 10 g) of each soil subsample was ground with a mortar and pestle, weighed, and loss on ignition (450°C for 12 hrs) was used to calculate carbon content assuming a 0.58 C fraction (Pouyat et al. 2002). Soil bulk densities were multiplied by the percent carbon of respective subsamples to calculate soil carbon mass.

#### Statistical analysis

A stepwise model selection procedure was used to determine which lawn management and climate proxy parameters (i.e., aspect and slope) correlate with spatial (inter-lawn) variation in soil carbon mass. Soil subsamples taken from the same lawn were averaged for the analysis. Separate modeling analyses were conducted on lawns with coefficients of variation < 0.25 for soil carbon mass and on all lawns to determine how within-lawn variation affected model explanatory power. Lawn management and climate proxy parameters were retained in the regression model when alpha  $\le 0.15$ , the default for the stepwise procedure in SAS statistical software (SAS Institute, Cary, NC, USA) and a commonly accepted alpha for regression modeling analysis (Montgomery et al. 2001).

Two-tailed t-tests were used to compare mean bulk density, soil carbon percent, and soil carbon mass between lawn and forest soils. A Wilk-Shapiro Normalilty test revealed that data were normally distributed and required no pre-analysis transformation. ANOVA with LSD was used to assess differences in soil carbon mass among self-ascribed environmental behaviors. All statistical analyses were conducted using SAS v. 9.1.

#### RESULTS

#### Lawn and forest soil properties

All residential lawn soil properties surveyed differed significantly from those of forest soils (P < 0.0001, Table 1). Forest soil bulk density in the top 10 cm was less than half of that observed in lawns, but soil carbon percent in forest soils was over 4 times greater than that of lawns (P < 0.0001, Table 1). This resulted in 67 % greater soil carbon storage in the top 10 cm of forest soils (Table 1).

TABLE 2. Relationships between lawn management practices and orientation, and soil carbon mass (to a depth of 10 cm) in an exurban neighborhood near Richmond, VA. Parameters were selected using a stepwise model procedure ( $\alpha = 0.15$ ).

	Lawn Management Practice or Physical Indicator	Effect on Soil Carbon Mass	Partial r <sup>2</sup>	Р
1.	Watering frequency during growing season	1	0.18	0.06
2.	Southeast facing orientation of lawn	1	0.11	0.11
3.	Fertilization frequency during	ļ	0.10	0.11

#### Lawn management, orientation and soil properties

Stepwise model selection indicated that soil carbon mass was significantly correlated with the frequency of common lawn management practices, watering (P = 0.06) and fertilization (P = 0.11), and with lawn cardinal orientation (P = 0.11). Higher frequencies of lawn watering and fertilization during the growing season corresponded with lower soil carbon mass in the top 10 cm (Table 2). Lawns that were oriented toward the southeast also had lower soil carbon mass than those facing northwest.

Based on these modeling results, an integrated management and orientation index was developed for predicting inter-lawn variation in soil carbon mass to a 10 cm depth. Discrete points were assigned to lawns with higher watering and fertilization frequencies during the growing season, and to those more closely oriented in the southeastern facing direction (Table 3). Thus, a high index value indicates greater lawn management intensity and an orientation toward a putatively dryer, warmer southeastern face. This index, when fitted against soil carbon mass using an exponential decay function, explained 57 % (P = 0.0006) and 18 % (P = 0.05) of the variation in soil carbon mass among lawns with low within-site variation (C.V. < 0.25) and among all lawns, respectively. Soil carbon mass in the top 10 cm exhibited a rapid decline from 4.5 kg m² in lawns with low indexes to a near asymptotic low of 2.8 kg m² in lawns with moderate to high indexes (Figure 2).

#### Environmental attitudes and soil carbon mass

Self-ascribed alignment with environmental issues was a moderate indicator of soil carbon mass (Figure 3). Survey respondents who claimed to be strong environmentalists had lawns with significantly greater soil carbon mass by  $0.8~{\rm kg}~{\rm m}^{-2}$  and they spent one hour less per week on lawn work than those who said that they agree with environmentalists on most issues. Statistical differences among other respondent categories were not significant (P > 0.1). Only one respondent claimed to not be an environmentalist at all and, because of insufficient replication, was excluded from statistical analysis.

TABLE 3. Point assignments used to calculate lawn management and orientation indexes for individual lawns. Parameters were selected for the index using a stepwise modeling procedure when  $\alpha < 0.15$  (see Table 2). Lawn indexes were calculated for each surveyed household by summing points associated with each parameter.

Parameter	Point assignment
Watering frequency during growing season	Never = 0; Monthly = 1; Weekly = 2; Daily = 3
Fertilization frequency during growing season	Equals number of fertilizer applications following manufacturer specification (0 to 4)
Cardinal orientation facing lawn	Northwest (270-360°) = 0; Northeast (0-90°), Southwest (180-270°) = 1; Southeast (90-180°) = 2
Total possible points	9

#### DISCUSSION

Results from this study show that land-use conversion from forest to lawn significantly reduced carbon storage in the top 10 cm of soil. Forest soil carbon storage reported in this study of 5.5 kg m<sup>-2</sup> in the top 10 cm is similar to 5 kg m<sup>-2</sup> reported for a suburban forest in Baltimore (Pouyat et al. 2002). The mean residential lawn soil carbon mass of 3.3 kg m<sup>-2</sup> in the top 10 cm is somewhat lower than that of other urban and suburban lawns of the eastern U.S. sampled to a 15 cm depth (Pouyat et al. 2002). Pouyat et al. (2009) observed comparable carbon storage in soils of >40-yr-old residential lawns and remnant urban forests of Baltimore city. The present study may have revealed lower soil carbon storage in lawns because land-use conversion from forest to exurban lawns was relatively recent (10 yrs), while older urban lawns of Baltimore have had substantially more time to accumulate carbon. Accumulation of soil carbon occurred for decades following land-use conversion from native habitat to residential lawns or golf course greens located in the arid western U.S. (Qian et al. 2003, Golubiewski 2006).

Results from the present study also indicate that lawn soil carbon storage declined with increasing management intensity. Lawn soil carbon storage in this study was negatively correlated with increased fertilization and watering frequency, and with a more southeastern facing lawn orientation. Empirical studies conducted in golf courses indicate mixed effects of fertilization on soil carbon storage, reporting either no effect (Qian and Follett 2002) or a positive effect (Higby and Bell 1999) of fertilization on soil carbon storage. Modeling studies uniformly predict a net increase in soil carbon storage with management intensification (Bandaranayake et al. 2003, Qian et al. 2003, Milesi et al. 2005). Findings from these empirical and modeling studies provide important quantitative assessments of how management behavior might affect soil

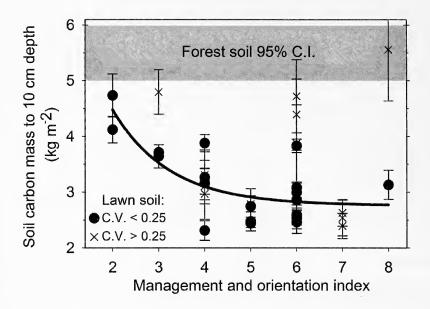


FIGURE 2. Lawn soil carbon mass (to 10 cm depth) in relation to integrated management intensity and lawn orientation indexes (see text and Table 3 for details), and in comparison to soil carbon mass of surrounding forest fragments. A higher index indicates greater lawn management intensity and/or a more southeastern facing orientation. Regression analysis was conducted using mean lawn soil carbon mass values with coefficient of variations (C.V.) < 0.25 (black filled circles; n = 21). Means with C.V. > 0.25 are also shown (black X's; n = 12). Gray-shaded area is the 95 % confidence interval of forest soil carbon mass (n = 24).

carbon storage; however, results from the present study suggest that residential ecosystems, which encompass a range of complex management behaviors, may not uniformly respond to common management practices in the same way.

Declining soil carbon storage with higher fertilization and watering frequency will occur if these parameters cause soil carbon inputs to decrease or carbon outputs to increase. Soil carbon storage decline is unlikely to be caused by a reduction in carbon inputs with fertilizer and water amendments because these supplements typically increase lawn primary production (Higby and Bell 1999, Qian et al. 2003, Milesi et al. 2005). Contrastingly, water and fertilizer amendments may stimulate microbial decomposition of soil organic matter, thereby increasing carbon losses from soils (Kaye et al. 2005, Rodriguez et al. 2005). Although the present study did not detect relationships between aeration and soil carbon storage, tilling and aerating stimulated soil organic matter decomposition in agricultural soils (Reicosky et al. 1997, Kandeler et al. 1999, Paustian et al. 2000). High management intensity in residential ecosystems

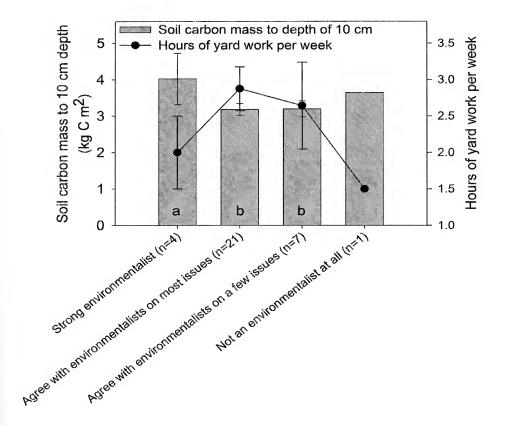


FIGURE 3. Soil carbon mass (10 cm depth) in relation to household views on environmental issues and to hours invested in yard work during the growing season. Survey respondents (n = 33) were asked: "How would you describe yourself with respect to environmental issues?" and "How many hours per week, during the growing season, do you spend caring for your lawn?" Letters indicate significant differences among means ( $\alpha = 0.05$ ).

is a putative cause for elevated microbial activity, and consequently decomposition rates, relative to surrounding natural ecosystems (Green and Oleksyszyn 2002, Koerner and Klopatek 2002, Kaye et al. 2005). In the present study, the mechanistic cause of declining soil carbon with increasing management intensity is unknown, but may be due to enhanced microbial activity. No other management behaviors (e.g., leaving

clippings onsite) were significantly correlated with soil carbon storage in the present study and autocorrelation among management behaviors was not detected.

Soil carbon inputs and outputs are also constrained by microclimatic conditions (Bandaranayake et al. 2003, Milesi et al. 2005), which likely varied according to lawn orientation in the present study. Lower soil carbon storage in lawns facing southeast may be caused by dryer, warmer microclimates, which could concurrently reduce net primary production and increase temperature-limited rates of microbial decomposition of organic matter in lawns that are well-watered (Wythers et al. 2005, Del Grosso et al. 2008). Additional investigation is required to quantify the balance of carbon inputs and outputs to residential soils. Particularly, quantitative assessments of carbon outputs are needed for residential ecosystems since most studies have investigated the contribution of carbon inputs to soil carbon storage (Kaye et al. 2006).

Findings from the present study provide novel support for the notion that social indicators can be useful, integrated predictors of soil carbon storage in residential ecosystems. Households that claimed to be more supportive of environmental issues stored significantly more carbon in their lawns (Figure 3), possibly because they spent less time managing their lawns in a way that may reduce soil carbon storage. Strong environmentalists, for example, spent less time managing their lawns and, consequently, watered and fertilized less frequently, behaviors negatively correlated with soil carbon storage in the present study. Results from this study are supported by a limited number of reports that show social indicators are robust predictors of ecosystem properties in human-dominated systems. For example, Tratalos et al. (2007) showed that demographic indicators of social status correlate with residential carbon storage rates in the United Kingdom. Other studies examined how lifestyle behavior or social status relate to parameters known to affect carbon storage, including vegetation cover and tree density (Grove et al. 2006) and fertilizer application rates (Robbins et al. 2001). Qualitative and semi-quantitative social indicators are promising predictors of ecosystem function in human-dominated ecosystems and may be important components of future "carbon footprint" models for urban areas; however, substantial additional research is required to determine which social indicators are the best predictors of residential soil carbon storage and to determine whether management attitudes and behaviors are causally linked (Whitford et al. 2001, Pataki et al. 2006, Grimm et al. 2008).

Results from the current study show that a simple model for estimating soil carbon storage in residential ecosystems may hold future promise, but predictive power is presently limited by unexplained spatial variability in soil carbon mass. High within-lawn variation in soil carbon storage limited the detection of strong statistical relationships with management behavior and orientation when all lawns were included in model development. Soil carbon storage was significantly correlated with the integrated index even when all lawns were included in the regression analysis ( $P \le 0.05$ ), but this caused a substantial decline in the model's explanatory capabilities. It is also important to note that this study is of a single neighborhood and, although this approach best addressed study objectives, results are limited in inference to ecosystems with similar social (e.g., economic) and physical (e.g. soils) dimensions. Despite these limitations, this study suggests that the general approach employed herein could be successfully modified to incorporate additional putative explanatory variables that aid in the development of more robust predictive models.

#### CONCLUSION

As residential ecosystems grow in number and area, numerous calls have been made to understand how human behavior modifies important ecosystem functions, such as carbon cycling (Vitousek et al. 1997, Pickett et al. 2005, Pataki et al. 2006, Liu et al. 2007, Pouyat et al. 2007, Grimm et al. 2008). Vitousek et al. (1997) asserted that contemporary ecosystem processes cannot fully be understood without investigating how and why humans interact with surrounding ecosystems.

This study is the first conducted in the Piedmont region of the southeastern U.S. to show that household lawn management is a significant predictor of soil carbon storage in residential ecosystems. Further investigation is warranted to evaluate why lawn management intensification decreased lawn soil carbon storage in the present study, a result that departs from some experimental and modeling studies conducted in other geographic regions. A broader understanding of household effects on carbon cycling in residential ecosystems will have implications for ongoing educational campaigns that seek to modify human behaviors that affect greenhouse gas emissions (Dilling 2007a).

#### ACKNOWLEDGMENTS

Chris Gough and Eliza Fritz worked jointly to develop methods described in this manuscript. Eliza, a Clover Hill High School student, conducted most of the data collection, soil processing, and preliminary data analysis and summary. Chris performed the final data analysis and manuscript preparation.

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#### Dear VAS Member:

The VAS Fall Meeting for Undergraduate Research will be held on October 16<sup>th</sup> at the Science Museum of Virginia. At this meeting, five \$500 research grants will be awarded to undergraduate students to support their research during the 2010-11 academic year. In order to be eligible for a research award, the student(s) must submit a brief research proposal by October 1, 2010 to:

Dr. Michael Renfroe Dept. of Biology, MSC 7801 James Madison University Harrisonburg, VA 22807.

The faculty mentor for the project must be a member of the Virginia Academy of Science by the October 1st deadline for submission of the grant application. The students are required to attend the VAS Fall Undergraduate Research Meeting and present a poster outlining their proposed research. Both the research proposal and the poster presentation will be evaluated to determine the recipients of the research awards. The application for the Undergraduate Research Grants is attached and is also available on the Virginia Academy of Science web site, www.vacadsci.org. In addition, the recipients of the research awards will present the results of their work at the VAS Annual Meeting in May.

As I indicated above, this year the fall meeting will be held on October 16<sup>th</sup> at the Science Museum of Virginia, 2500 West Broad Street, Richmond. The poster session will be held in the morning followed by lunch, guest speaker and announcement of the research awards.

We are very excited about this program and hope that you will encourage your undergraduate research students to participate. Also, please pass this information on to other faculty at your institution who sponsor research students and encourage them to become members of the Academy and to participate in this program. Help us to make this a successful program and one that we can expand in the future.

Sincerely, Michael Renfroe, Chairman Fall Meeting Committee

## 196 FALL UNDERGRADUATE RESEARCH MEETING

# VAS Fall Meeting for Undergraduate Research SCHEDULE OF EVENTS Saturday, October 16, 2010

9:00	arrival time
9:00-10:00	poster set-up and coffee hour
10:00-12:00	evaluation of posters (During this time period, each poster will be evaluated by a team of judges. The judges will meet with each student and the student should be prepared to give a brief presentation (no more than 5 minutes) on the proposed research and to answer questions from the judges.)
12:00-1:00	lunch
1:00 - 2:00	Invited Speaker
2:00 - 2:30	announcement of grant recipients
2:30 - 3:00	remove posters and depart

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Virginia Journal of Science Volume 61, Number 1& 2 Spring/Summer 2010

# Garlic as an Alternative Anthelmintic in Sheep A. Curry and B. D. Whitaker<sup>1</sup>

Agriculture Program, Ferrum College, Ferrum VA, 24088, USA

#### **ABSTRACT**

An increase in the anthelmintic resistance of parasites in small ruminants and a push towards non-chemical farming has lead researchers to search for alternative forms of anthelmintics. The efficacy of treating sheep by using natural garlic extract with respect to changes in weight, fecal egg counts (FEC), and packed cell volume (PCV) was investigated. Sheep were treated every 4 weeks for 8 weeks with saline (control), Ivermectin, or natural garlic extract and samples were collected weekly to determine FEC and PCV. Sheep were weighed at the time of sample collection to determine weight change. There were no significant differences between the Ivermectin and natural garlic extract treated sheep with respect to weight changes, FEC or PCV. The Ivermectin and natural garlic extract treated sheep had significantly greater (P < 0.05) weight changes and PCV compared to the control. The control sheep had a significantly higher (P < 0.05) FEC compared to the Ivermectin and natural garlic extract treated sheep. Administering natural garlic extract as anthelmintic is a variable alternative to Ivermectin.

#### INTRODUCTION

Small ruminant gastrointestinal nematodes potentially reduce production and cause profit loss throughout the sheep industry (Perry and Randolph, 1999). Traditionally, producers have used commercially available anthelmintics (benzimidazoles, imidazothiazoles-tetrahydropyrimidines, and avermectins-milbemycins) to control parasites. However, an increase in the anthelmintic resistance of parasites is currently a serious threat and research is being done to find alternative forms of anthelmintics (Larson, 2006). The societal push towards non-chemical (eco-friendly, green, organic) farming has also hastened the search for viable alternatives to chemical anthelmintics (Waller and Thamsborg, 2004).

Haemonchus Contortus, often referred to as the "barber pole" worm, punctures the lining of the abomasum, causing blood plasma and protein loss in sheep. Since it is usually the most prevalent nematode parasite in sheep and causes the most destruction, a majority of the research in alternative forms of anthelmintics has been targeting H. Contortus. Copper oxide wire particles administered to sheep have been shown to reduce the number of H. Contortus strongyle eggs shed in lambs (Burke et al., 2004) as well as pregnant ewes (Burke et al., 2005). Developing vaccinations against parasites is becoming a reality with the use of recombinant protein-based vaccines (Knox, 2000), however the economical availability of such vaccines to producers is

<sup>&</sup>lt;sup>1</sup> Corresponding Author: B. D. Whitaker, University of Findlay, 1000 North Main Street, Findlay OH, 45840, USA. E-mail: whitaker@findlay.edu

currently not a possibility.

Additional research has shown that feeding forages high in tannin content reduces the fecal egg counts (FEC) and number of worms in goats (Shaik et al., 2006). Strong evidence suggests that using chlamydospores (nematode ingesting fungi) as an anthelmintic in sheep is very successful (Fontenot et al., 2004) however it has not been shown to target *H. Contortus*. Although not an alternative treatment, research indicates that breeding and selecting for ewes with higher genetic merit for growth as lambs, and lambs with higher genetic merit for body weight, were all more resistant to infection as adults (Vanimisetti et al., 2004).

Any novel treatment needs to easily be incorporated into a producers flock management and be economically feasible (van Wyk et al., 2006). The current techniques available to test the efficacy of any treatment are serology testing, the FAMACHA chart based on the color of the eye membrane mucosa (measures relative levels of anemia) (van Wyk and Bath, 2002), and FEC (Cringoli et al., 2004). Novel treatments and management systems should be able to be evaluated using the above techniques.

Therefore, the objectives of this study were to compare the use of natural garlic extract to Ivermectin as an anthelmintic for sheep with respect to, 1) change in body weight (BW), 2) FEC, and 3) packed cell volume (PCV) based on serology analysis.

#### MATERIALS AND METHODS

**Experimental Site and Treatment Groups** 

This study was carried out at the Ferrum College Agriculture Center in Ferrum, Virginia (36°92'N), between May and September 2009. The experimental site was at an altitude of 430 m and consisted of a 0.5 ha plot containing a clean fescue and orchard grass mix. Free access to unlimited water was available throughout the study.

A total of 14, 90-d sheep (American black-faced) ranging from 18 to 32 kg (average = 26 kg) were randomly assigned to receive orally either saline (control), natural garlic extract (Garlic Barrier, Glendale CA, 1 teaspoon/head), or Ivermectin (Merial Ivomec, Atlanta GA, 42 mg/kg bodyweight). All sheep were treated on week 0 and week 4 of the 9 week study. Samples were collected each week for analysis.

#### **Body Weight Measurements**

Sheep were weighed weekly using an electronic scale (A and A Scales LLC, Prospect Park, NJ, USA) to monitor changes in BW.

#### Fecal Egg Counts

Feces were taken rectally using the gloved hand method and stored at 4° C until analysis. All FEC were performed using the McMaster method (Cringoli et al., 2004). Briefly, approximately 4.0 g fecal material was placed in a 50 mL graduated cylinder and Sodium Nitrate (1.2 – 1.25 specific gravity) was added to bring to final volume to 26 mL. The solution was homogenized, filtered through 2 layers of cheesecloth, and immediately 1.0 mL of resulting solution was placed on a McMaster slide. The slide was incubated at room temperature for 5 min to allow the eggs to float to the top of the solution and strongyle eggs were counted at 100X using a compound light microscope.

#### Immune Response

Whole blood was obtained via jugular venipuncture (BD Vacutainer, 12 mg EDTA, Franklin Lakes, NJ) at stored at 4°C until analysis. The PCV was determined using microfuge hematocrit tubes.

#### Statistical Analysis

The experiments were set up as complete randomized designs and the data were analyzed using the general linear model (GLM) procedure in SAS (SAS Institute, Cary, NC, USA). The effects included in the model were treatment and time. There was no significant effect for time and it was dropped from the final model. Significance between treatments was analyzed using the least-square means (LSMEANS) statement with the possible probability values (PDIFF) options. In all analyses, P < 0.05 was considered significant. Results are expressed as the least-squares mean  $\pm$  s.e.m.

#### RESULTS

#### **Body Weight Measurements**

There was no significant difference between the change in BW of the natural garlic extract treated sheep  $(0.23 \pm 0.17 \text{ kg})$  when compared to the Ivermectin treated sheep  $(0.36 \pm 0.15 \text{ kg})$ . The natural garlic extract and Ivermectin treated sheep were significantly heavier (P < 0.05) than the control sheep  $(-0.26 \pm 0.17 \text{ kg})$  (Figure 1).

#### Fecal Egg Counts

There was no significant difference between any of the treatment groups at the first week, prior to treatment administration. Throughout the remainder of the study, there was no significant difference in the FEC of the natural garlic extract treated sheep (1805.0  $\pm$  613.0 eggs/g feces) when compared to the Ivermectin treated sheep (863.0  $\pm$  548.0 eggs/g feces). The control sheep had significantly higher (P < 0.05) FEC (4993.0  $\pm$  613.0 eggs/g feces) than the natural garlic extract and Ivermectin treated sheep (Figure 2).

#### Immune Response

There was no significant difference in the PCV between any of the treatment groups at the first or second week of the study. Throughout the remainder of the study, there was no significant difference in the PCV of the natural garlic extract treated sheep (25.5  $\pm$  1.1 %) when compared to the Ivermectin treated sheep (27.5  $\pm$  1.0 %). The natural garlic extract and Ivermectin treated sheep had significantly higher (P < 0.05) PCV than the control sheep (18.9  $\pm$  1.1 %) (Figure 3).

#### DISCUSSION

The results presented in this paper compare the use of a commercially available anthelmintic (Ivermectin) to the use of a non-chemical alternative (natural garlic extract) in the parasite management of sheep. As sheep producers continue to see an increase in anthelmintic resistant parasites in their flocks, there is an increased demand to find alternatives to the current practices (Larson, 2006). Our results indicate that by administering natural garlic extract (Garlic Barrier) orally using the same time guidelines as the commercial anthelmintics there were no significant differences in BW, FEC, and PCV compared the commercially treated sheep.

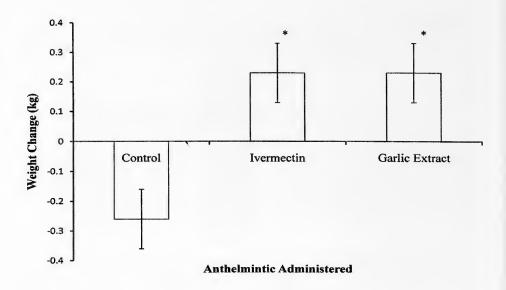


FIGURE 1. Effect of anthelminitic administered every 4 weeks on weight change (kg) after 8 weeks. Data are expressed as mean  $\pm$  SEM. Means with different superscripts differ at least P < 0.05.

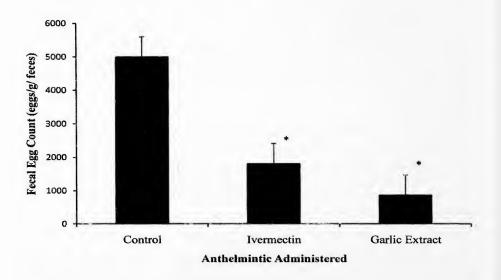


FIGURE 2. Effect of anthelmintic administered every 4 weeks on fecal egg count (FEC) (eggs/g feces) after 8 weeks. Data are expressed as mean  $\pm$  SEM. \*Means with different superscripts differ at least P < 0.05.

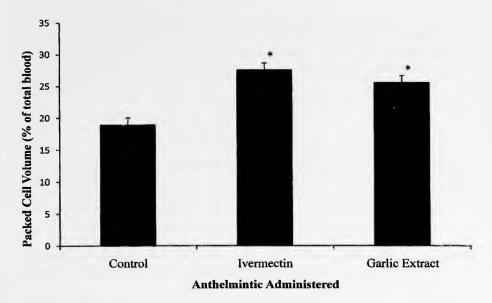


FIGURE 3. Effect of anthelmintic administered every 4 weeks on packed cell volume (PCV) (% of total blood volume) after 8 weeks. Data are expressed as mean  $\pm$  SEM. Means with different superscripts differ at least P < 0.05.

Throughout the experiment we observed that there was no significant change in BW between sheep that were administered natural garlic extract and those that were administered Ivermectin. The sheep that were not treated with an anthelmintic lost weight compared to the other treatment groups, most likely due to the increase in parasite load. This is advantageous to the producer because they could implement using only natural garlic extract instead of the commercially available anthelmintic (Ivermectin) and still achieve the same rates of production (Perry and Randolph, 1999).

We observed the same trend with the PCV in response to treatment groups. We believe that the low PCV in the untreated group was due to an immune response. Elevated levels of *H. Contortus* in the abomasums can cause a decreased immune response thus leading to a loss in blood plasma, serum protein, and decreased PCV (Scharenberg et al., 2008). Our results are in agreement with previous studies that show not treating sheep with anthelmintics causes a decrease in PCV and the symptoms can be eliminated by treating with commercially available anthelmintics (Yacob et al., 2009).

Little scientific research has been conducted on using garlic as an anthelmintic in sheep, but it is thought that garlic does not prevent the production of parasite eggs but rather the hatching of eggs into larvae (Bastidas, 1969). Our results are in agreement with this hypothesis, as we observed no difference in FEC between the garlic extract and commercially available anthelmintic treatment groups.

Our results indicate that using garlic extract as an oral anthelmintic in sheep is a viable option to commercially available chemicals. Further research still needs to be done to determine if there is a dose response and the most economical dose without losing its effect. A better understanding of the specific mechanism of action of garlic in the animal and the immune system could lead to future solutions to controlling anthelmintic resistant parasites in small ruminants.

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# ABSTRACTS OF PAPERS, 88th Annual Meeting of the Virginia Academy of Science, May 20 - 21, 2010, James Madison University Harrisonburg, VA

# **Aeronautical and Aerospace Sciences**

TRACING THE GROWTH OF THE U.S. NAVY AVIATION. M. Leroy Spearman. Langley Research Center, Hampton, VA & Robert W. Heath, RRMC, Newport News, VA. The Navy's first interest in aviation may have occurred in March 1898. Assistant Secretary of the Navy, Theodore Roosevelt, impressed by Samuel P. Langley's success with flying models, reported the potential of aviation for use in war. A joint board (Navy & Army) was appointed to consider the role of aircraft in warfare. The board's report expressed a view in favor of aviation for military purposes. The Navy did acknowledge a role for aviation in September 1910 when Capt. Washington I. Chambers was designated as the officer in charge of aviation matters. Soon Glenn Curtiss, a U.S. pioneer in aviation, began meeting with Chambers in an effort to convince the Navy of the value of aircraft for Naval use. In November 1910 Eugene Ely took off in a Curtiss airplane from a wooden platform built over the bow of a ship in the harbor at Hampton Roads, VA. In 1921 the Army Air Service conducted bombing tests in which bombers sank a captured German battleship. The Navy recognized the need for fleet defense and expanded the development of aircraft carriers. In March 1922 at Norfolk, VA, the Navy commissioned the USS Langley (CV-1), the first aircraft carrier, on which the first take off and landing and the first catapult launching were made. In 1933 the first ship designed as an aircraft carrier, the USS Ranger (CV-4), was launched at the Newport News ship yard. Since then, 28 aircraft carriers have been constructed for the Navy at that shipyard and Navy aviation was well underway.

SOME CONTRIBUTIONS TO AERODYNAMIC RESEARCH FROM THE NACA/NASA. M. Leroy Spearman. NASA-Langley Research Center. Hampton, VA & Katie Klein, MITRE Corp. McLean, VA Leonardo da Vinci envisioned man-flight in the 15<sup>th</sup> century and designed a practical airplane concept in 1490. Many other pioneers proposed various types of flying machines over the next 400 years but it was not until!903 that the Wright Brothers, were credited with achieving the first manned-powered flight. The use of aircraft by European nations in World War I lead to an act of the U.S. Congress in 1915 that established the National Advisory Committee for Aeronautics (NACA). Thus research began at Langley Field, VA in the early 1920's. This research has transformed low-speed, wood and fabric, propeller-driven airplanes into high speed, all-metal, jet-propelled airplanes. In July 1955 the U.S. announced plans to launch an earth-orbiting satellite. The need for space research lead to the establishment of the National Aeronautics and Space Administration (NASA) in July 1958. The nucleus of the NASA was the existing NACA with the charge expanded to include space research. The skilled researchers at NASA-Langley have continued to provide improvements in aircraft

developments and contribute to the development of spaceflight as well. Continued advances in aerospace research required well trained researchers. To this end, NASA-Langley participates in mentorship programs to encourage high school students to become researchers. The first author of this paper has been a mentor for this program and the second author of this paper has been a student.

FEDERAL FUNDING OF AERODYNAMIC RESEARCH IN THE UNITED STATES. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA 23681. In 1898 the Assistant Secretary of the Navy, Theodore Roosevelt reported to the Secretary of the Navy of the potential of aviation for use in war. A joint board was appointed (Army & Navy) to consider the role of aircraft in warfare. In April 1898 the Board's report favored the value of aviation for military purposes. In 1910 Capt Washington I. Chambers was designated officer in charge of Navy aviation matters. The Navy constructed an Experimental Model Basin. In 1912, at the urging of Capt. Chambers, President Taft appointed a board to consider a national aerodynamic laboratory. Legislation to create the laboratory was introduced in Congress in 1913 but was defeated. When war broke out in Europe in 1914 it was apparent that European countries had adapted the airplane and that development in the U.S. was lagging. Charles D. Walcott of the Smithsonian Institute undertook the effort to get approval for a federally funded aerodynamics laboratory. In January 1915, Assistant Secretary of the Navy, Franklin D. Roosevelt endorsed a resolution introduced in Congress for the creation of an advisory committee for aeronautics and suggested that it be attached to the Navel Appropriations Bill. The bill was approved on March 3, 1915. President Woodrow Wilson signed a bill that established the National Advisory Committee for Aeronautics (NACA), now the National Aeronautics and Space Administration (NASA), that provides federally funded research for aeronautics and astronautics.

# Agriculture, Forestry and Aquaculture Science

EFFECTS OF DIFFERENT ORGANIC APPLICANTS ON SOIL CONDITIONS FOR BLUEBERRY PRODUCTION. Jeremiah D. Vallotton & Roman J. Miller, Dept. of Biology, E. Mennonite University, Harrisonburg VA, 22802. Blueberries are a potentially profitable crop that have yet to be tested under organic agricultural practices in the Shenandoah Valley of Virginia. Blueberries require a low pH between 4.8 to 5.5 and high levels of organic matter for optimal growth. In this experiment, four treatments of organic matter (horse manure, sheep manure, pine needles, and compost) were used to grow blueberries, along with a control plot where chemical herbicides and pesticides will be used. Elemental sulfur was used on all plots to lower the pH of the soil over time. Soils were sampled and analyzed to measure soil pH, soil organic matter, and nutrients. Tests suggest that the organic treatments had a highly positive effect on the soil organic matter levels, while decreasing the pH yielded higher levels of available Mn and Fe, but Cu remained low. Soil pH mostly decreased to desirable ranges, but in some of the plots application of sulfur resulted in less change. The most recent tests indicate that the pH continued to decrease, from an average of 6.1 to 5.5,

though the manure treatments were still somewhat high. Organic matter increased by 19%. Organic amendments provide excellent conditions for successful organic production of blueberries. (Research supported in part by USDA Specialty Crop Grant # 2008-427, Commonwealth of Virginia, Department of Agriculture and Consumer Services.)

ACTH 22-39 INDUCES HYPERPHAGIA AND ANXIO-LIKE BEHAVIORS IN COBB-500 CHICKS. Caitlin A. Reid, Evin L. Guilliams, and Marissa L. Smith. Dept. of Biol., Radford University, Radford, VA 24141. The melanocortin system plays a key role in the regulation of appetite in both mammals and birds. A member of this system, ACTH 22-39, is cleaved from proopiomelanocortin and is a well known insulin secretagogue. However, it also increases food intake in mammals. To our knowledge its effects are unreported in avians. When centrally administered, ACTH 22-39 (0, 2, 4, 8 nmol) increased food intake in fed 4-day post hatch Cobb-500 chicks but did not affect whole blood glucose concentrations. That ACTH 22-39 is an insulin secretagogue and food intake increases glucose may explain this lack of a net effect on glucose concentrations. To determine if food intake was competitive with other behaviors a 60 min behavioral analysis was conducted which revealed that ACTH 22-39 also increases the number of jumps and escape attempts. Other behaviors were not affected. These data may lead to novel treatments for anorexia in other species, including humans.

COMPARATIVE GENOMICS - ANALYSIS OF GENETIC **FACTORS** INFLUENCING RESISTANCE TO GASTROINTESTINAL PARASITES. Damarius Fleming & Glenn C. Harris, Dept. of Biology, Virginia State University, Petersburg, VA 23806. Gastrointestinal (GI) parasite infections are a primary limitation to livestock production. As nematode resistance to anthelmintics continues to spread worldwide the search for factors that confer genetic resistance gains new importance. We use a mouse model as a framework for comparative genomic mapping with cow, rat, chicken and human to identify the genetic factors associated with phenotypes that are resistant to GI infections. A total of 10 regions in mice were matched with syntenic regions in the other species and an accumulated list of candidate genes was assembled and prioritized. Most candidate genes identified have a function involving cytokine function or intestinal immune regulation. These results confirm that GI nematode response is multifactorial, potentially including the interaction of many genes, and provide a list of prioritized gene candidates that represent prime targets for individualized functional analysis in future studies.

DEVELOPMENT OF DNA PROBES FOR DETERMINATION OF SPECIES IN MIXED POPULATIONS. Tiffany Toledo and Brian L. Sayre, Dept. of Biol., Virginia State Univ., Petersburg, VA 23806. A quick reliable test to determine the species of organisms found in environmental samples has far reaching potential. An example may be to determine the source of contamination in a ground water location during a disease outbreak. We are developing a technique to use DNA for determination of individual species in a mixed species environment. The objective of this experiment was to

develop a DNA probe for use in this technique. The DNA of mixed plant samples was amplified using PCR with primers developed for the NADH dehydrogenase subunit 5 gene of the mitochondrial DNA. The PCR product was attached to an Au nanoparticle and DNA binding determined with spectrophotometric anlayses. Ultimately, these nanoparticle-DNA probes will be used to create a detection system that will have increase sensitivity and capability to detect DNA from multiple organisms in an environmental population.

HOW TEMPERATURE AND LIGHT AFFECT TOMATO YIELD IN WINTER-SPRING GREENHOUSE PRODUCTION. Mark E. Kraemer and Françoise Favi, Agricultural Research Station, Virginia State University, Petersburg, Virginia 23806. Rising energy costs have been a significant factor in winter-grown greenhouse tomato Propane prices generally track the cost of oil. It was proposed that reducing temperatures in the greenhouse during periods of cloudy weather could reduce costs with little to no loss in tomato production. Previous research indicated that under low light conditions (PAR<100µE) the photosynthetic rate of variety Trust leaves, the most commonly grown greenhouse tomato used in the mid-Atlantic region, was not significantly different at 64°F than at 74°F. In this research, two sections of a glass greenhouse were used to compare tomato yield under different temperatures. Daytime temperatures in the west section were set to 74°F whereas east section was set to 64°F, before fans would come on. Night temperatures were the same in both sections (60°F). The overall difference in temperature between the two sections was 2.3°F. After 8 weeks, the warm section yielded 27% more ripe fruit than the cool room. However, the total weight of tomato fruit (harvested and green on the vine) was the same in the two sections. It appears that warmer temperatures do not increase tomato fruit biomass but promote fruit ripening. Because fruit biomass is not lost with cooler temperatures under low light conditions it may be possible to use temperature to time fruit production to market needs, or to save energy during periods of unusually cold weather.

INFLUENCE OF SHADOW COSTS OF FRESHWATER SHRIMP PROUCTION IN VIRGINIA. Brian Nerrie, Virginia Cooperative Extension, Virginia State Univ., Petersburg VA 23806. Consumers in the United States eat more shrimp than any other seafood. A great majority of shrimp are imported or harvested by domestic fishers in areas such as the Gulf of Mexico. Freshwater prawn (Macrobrachium rosenbergii) farming is presently expanding in Virginia. The prawn not only has established its own market, but has been shown to be an excellent substitute for marine shrimp. During 2009 prawn selling prices ranged from \$15.40 - 22.00/kg. Production averaged between  $500 - 1000 \, \text{kg/ha}$  and net returns ranged from \$7700 - 22,000/ha. A producer association was subsidizing some input costs. Shadow costs, the maximum price a farmer is willing to pay for an extra unit of input, were examined for two major production inputs: sinking feed and juvenile shrimp. Feed cost (\$0.66/kg) would have to increase by more than 100% to impact on the decision to produce. Juvenile shrimp costs would have to increase from \$0.06/shrimp to more than \$0.17/shrimp to discourage production.

USE OF WETLANDS TO REDUCE TROUT PRODUCTION EFFLUENT NUTRIENTS. Bryan Taliaferro & Brian Nerrie, Dept. of Agriculture, Virginia State Univ., Petersburg VA 23806. Wetlands are among the most productive ecosystems in the world that can filter nutrients, sediment, and pollutants from surface and ground water, absorb excess floodwater and rainwater, and provide habitats to numerous plants and animals. Constructed wetlands are engineered marshes that duplicate natural processes to cleanse water, process livestock effluent, human waste, and drainage water. With the worldwide demand for aquaculture products increasing, production of trout requires substantially high amounts of quality inflow water. This causes an increase in effluents that are discharged to the environment with enhanced nutrient and solid concentrations. Effluents contain significant amounts of organic and inorganic nutrients such as nitrogen and phosphate. Forms of nitrogen found are nitrogen - N2 nitrate -NO<sub>3</sub>, ammonium nitrogen - NH<sub>4</sub>. Wetlands can be used as a cost-saving alternative treatment method that can combine both mechanical and biological effluent treatment. Wetland plants can be used for their ability to absorb nutrients through surface or subsurface flow of water through the wetland. Certain plants can be found commonly around wetlands and make a greater contribution to the percent nutrient removal under low-load conditions than high-load. For wetlands that treat trout effluent with high loads of nitrogen (3.2 to 15.6g N m<sup>-2</sup>d<sup>-1</sup>) to wetlands with lower loads (0.4 to 2.0g N m<sup>-2</sup>d<sup>-1</sup>) <sup>2</sup>d<sup>-1</sup>), plants assimilated on 1 to 4% of the nitrogen in the higher loads, as opposed to 18 to 30% of nitrogen in the lower loaded systems. This is significant in that with in a low load trout production, a constructed wetland of a particular size with the incorporated plants can reduce the amount of nitrogen released into the environment. In conclusion, wetlands can be a less expensive and more environmentally efficient method of removing nutrients from trout effluent as well as other sources if necessary.

A NEW PROPOSED STANDARD WEIGHT EQUATION DERIVED FROM HISTORICAL WEIGHT-LENGTH DATA TO CALCULATE RELATIVE WEIGHT OF CHANNEL CATFISH FINGERLINGS. Edward N. Sismour, Agricultural Research Station, Virginia State University, P.O. Box 9061, Petersburg, VA 23806. A standard weight  $(W_s)$  equation for calculation of relative weight  $(W_r = \text{observed})$ weight /  $W_s$  x 100) of channel catfish, Ictalurus punctatus, grown in aquaculture is proposed. The function,  $\log W_s = -5.163 + 3.042 \cdot \log[\text{total length } (L_7, \text{mm})]$ , was derived using the RLP technique from nine weight-length relationships originally published as producer guidelines. It was compared to an equation published by Brown, Jaramillo, Gatlin, and Murphy (1995) for feral channel catfish and to Fulton's K using published mean weights-at-length of pond-raised catfish that received supplemental ration, observed weights-at-length of catfish grown in cages, and observed weights-atlength of feral catfish from the James and Rappahannock rivers, Virginia.  $W_{s < proposed}$ was  $\sim$ 50 percent higher at 7 cm and  $\sim$ 12 percent lower at 58 cm compared to  $W_s$ <established>  $W_{r < proposed}$  increased linearly and  $W_{r < established}$  decreased nonlinearly with  $L_T$ for catfish receiving supplemental ration. Among cage-grown catfish,  $W_{r \text{ proposed>}}$  and K showed no length-bias after three weeks. Among feral catfish,  $W_{r < proposed}$  showed no length-bias from 7 cm to 40 cm whereas  $W_{r < \text{established}>}$  decreased and K increased with  $L_T$ .  $W_s$  predicted by the proposed equation for channel catfish from 7 cm to 40 cm and

 $W_s$  predicted by the equation of Brown et al. for channel catfish greater than 40 cm are suggested as a single reference guideline for channel catfish producers in lieu of the variable guidelines that are currently available.

TROUT GROWTH UNDER ALTERNATIVE FEEDING LEVELS. Terrone Jaspers & Brian Nerrie, Dept. of Agriculture, Virginia State Univ., Petersburg VA 23806. Rainbow trout (Oncorhynchus mykiss) is the primary cold water aquaculture species in Virginia. Four 7000-liter outdoor tanks were stocked with equal size (80 g) and number of trout (50) on 10 March. Water in the tanks was provided with diffused air from a centralized air pump. Each trout population was fed a 36% crude-protein floating pellet at a rate of 50, 100, 150 and 200 g/day. The study was terminated after 30 days. Water quality parameters were monitored in each tank. Alkalinity was 30 ppm with the pH ranging from 7.5-8.5. Ammonia levels were not detectable. Dissolve oxygen concentrations were consistently greater than 5 ppm. High temperatures (>22°C during the fourth week of the study resulted in the termination. Feed conversion ranged from 1.5 to 1.9. Average sizes of trout at harvest were: 98g (fed 50g); 111g (fed 100g); 128g (fed 150g); 160g (fed 200g.)

IDENTIFICATION OF CANDIDATES GENES FROM SHEEP QTL REGIONS USING A SYSTEMS BIOLOGY APPROACH. Brian L. Sayre, Dept. of Biol., Virginia State Univ., Petersburg, VA 23806. Variability in the genomic regions defined by QTL studies for parasite resistance has hindered the identification of candidate genes for selection. A pathway analysis combined with a haplotype analysis across species can narrow the potential candidate gene list down significantly. Data from QTL regions were collected from published projects in sheep, cattle, mice, rats, and human. The complete gene list was compared to the KEGG pathway database. Identified genes from the pathways were compared to determine haplotype differences between the CBA and SWR strains of inbred mice, a known model for parasite resistance. The pathways identified were Fc epsilon R1 signaling pathway, focal adhesion, regulation of actin cytoskeleton, ubiquitin mediated proteolysis, MAPK signaling pathway, and pathways in cancer. Of 172 pathway genes belonging to at least one QTL data set, over half were also found in a QTL region in another species, and 10% were found in QTL regions from four of the five species. Comparisons of the CBA to SWR HapMap indicated that of the 40 possible SNPs, 20 SNPs were differentially identified in 15 genes between the model strains. This methodology has promise as a mechanism to improve the process of candidate gene identification from QTL regions. Improvement of the candidate gene identification process in turn will lead to increased identification of relationships among genes and economically important phenotypes.

# Astronomy, Mathematics and Physics (Including Materials Science)

STOCHASTIC STIFFINESS MATRIX AND MODAL ANALYSIS IN NON-DESTRUCTIVE DAMAGE DETECTION IN STRUCTURES. Anthony A. Teate, Department of Integrated Science and Technology, James Madison University, 800 S. Main St., Harrisonburg, VA 22807. We examine from first principles the effects of stochasticity in the stiffness matrix on the modal differential equations for a vibrating structure. We construct generalized stochastic model equations which describe the dynamical properties of the structure. The stochastic stiffness matrix is statistically described as a stationary, random, Gaussian stochastic process with zero mean. Closed form solutions of the equations are developed through calculation of all necessary moments. It is shown that a fluctuating stiffness matrix, and concomitantly the fluctuating frequency, can lead to an incorrect estimation of the frequency bandwidth of the frequency response function. This can result in an erroneous estimation of the damping and associated deformation in a structure. Similarly, the experimentally determined phase is shown to be in error when the effects of stochasticity in the frequency are neglected. We discuss the implications of these results on modal analysis when used in nondestructive testing and evaluation of damage in structures.

EVIDENCE OF A LONG-TERM WARMING TREND IN CHESAPEAKE BAY, VIRGINIA. William C. Coles<sup>1</sup>, Thomas C. Mosca III<sup>2</sup>, & Yohana K. Flores<sup>3</sup>, <sup>1</sup>Division of Fish and Wildlife - Department of Planning and Natural Resources - U.S. Virgin Islands, <sup>2</sup>Rappahannock Community College, Dept of Mathematics, 52 Campus Drive, Warsaw, VA 22572, & 3Rappahannock Community College, 52 Campus Drive, Warsaw, VA 22572. Water temperature data collected on the York River at Gloucester Point, Va. from 1954 until the 2002 were analyzed for trend. Water temperature is a sum of many functions, some periodic and some not. By collapsing the data into annual summer and winter means, the long-term trend was exposed. The trend was established to exist in the entire Virginia portion of the Chesapeake Bay by comparison with similar means of data collected on the tidal portions of the James, York, and Rappahannock Rivers, and the Chesapeake Bay over the years 1984 through 2008. The long-term trend indicates increasing temperatures, with a recorded change of 1.5 °C over the period of record. The warming trend is persistent across every location and time period examined. We thank Virginia Institute of Marine Science, Old Dominion University, and Virginia Department of Environmental Quality for the data used in this study.

ALGEBRAIC TORSION AND GENERAL RELATIVITY. Joseph D. Rudmin, Dept. of Integrated Sci. and Tech., James Madison Univ., Harrisonburg VA 22807. Ed Parker and James Sochacki have discovered a powerful way of finding polynomial approximations to systems of differential equations. This "Parker-Sochacki" or "Power-Series" method always finds the Taylor Series approximation to a given order, if such approximation exists, and has absolute error limits. For most practical applications, a Padé approximant derived from the Taylor Series provides better fit than

the Taylor Series. However, both Taylor Series and Padé Approximants have difficulty modeling poles in the solution. Often one can best model a pole by a change of variable, where the variable explicitly contains the pole. The change of variable can be found from the differential equations by eliminating the highest order feedback loop in the Parker Sochacki approximation, thereby simplifying those equations.

ALGEBRAIC TORSION AND GENERAL RELATIVITY. Joseph D. Rudmin, Dept. of Integrated Sci. and Tech., James Madison Univ., Harrisonburg VA 22807. Algebraic torsion offers a compact representation of Lorentz transformations, and contains the same symmetry as Dirac Spinors of quantum relativity, in a context that might be extended to general relativistic tensors, unifying the two theories. This talk elaborates on algebraic torsion as discussed in the book *Geometric Algebra and Applications to Physics* by Venzo De Sabbata and Bidyut Kumar Datta.

CALCULATING THE EXACT POOLED VARIANCE. Joseph W. Rudmin, Dept. of Physics and Astron., James Madison University, Harrisonburg VA 22807. An exact method of calculating the variance of a pooled data set is presented. Its major advantages over the many other methods are that it is simple, involves no assumptions, and is exact. The Exact Pooled Variance is the mean of the variances plus the variance of the means of the component sets." In this calculation, all means are averages weighted by the number of points in each data set. The statement will be proven, and the practical significance discussed. Please refer to this method as "the Exact Pooled Variance". This paper is available at:

http://csma31.csm.jmu.edu/physics/rudmin/PooledVariance.htm

COUPLED AND UN-COUPLED ORDINARY DIFFERENTIAL EQUATIONS: WHAT IS THE IMPLICATON? James S. Sochacki, Dept. of Math, James Madison Univ., Harrisonburg VA 22807. Let n, m be natural numbers. Let  $x \hat{\mathbf{l}} \mathbf{R}^n$  be a function depending on t and let  $f: \mathbf{R}^n m \to \mathbf{R}^n$  be a function on  $\mathbf{R}^n$ . Consider the autonomous system of n ordinary differential equations (ODEs) of order m given by  $x^m = f(x, x', x'', ..., x^{m-1})$ . If n=1 then we only have one ODE depending on the single variable x and we say the ODE is uncoupled from any other ODE. If n>1, but we can write the n ODEs as n uncoupled ODEs, then we say the system is uncoupled. Otherwise, we say the system is coupled. In this talk I will show how to uncouple a large class of ODES that are coupled. I will also discuss the mathematical, physical, and philosophical consequences of this surprising result.

DESIGN AND CONSTRUCTION OF A TWO-BEAM ASTRONOMICAL POLARIMETER. Gregory A. Topasna, Daniela M. Topasna, Gerald B. Popko, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. We present the progress made on the two-beam optical polarimeter that was

designed and constructed at Virginia Military Institute. We discuss the transition from laboratory testing and design to the construction of a portable working prototype currently being evaluated at the 20-inch telescope at the VMI observatory. Current results and capabilities of the instrument, as well as planned observations, will be presented

EXPLORING "THE GAME OF LIFE" IN SMALL WORLDS. Richard L. Bowman, Dept. of Physics, Bridgewater College, Bridgewater, VA 22812. While Conway's "Game of Life," an example of 2-D cellular automata, has been shown to have parallels with the biological world, researchers debate the role of boundary conditions in large universes on the patterns and behaviors observed there. This paper examines the effects of various boundary conditions on small worlds, 25 X 25 cells or smaller, and illustrates the dramatically different behavior resulting from an identical starting arrangement of cells (seed), the R-pentamino. The boundary conditions are referred to by their geometric analogs: torus, box, loop, and Möbius strip.

WHEN STEVEN HAWKING'S ALIENS ATTACK, A MATHEMATICAL MODEL. Yooryeon "Eddy" Jeon and Kristopher M. Kalish, Dept. of Mathematics and Statistics, James Madison University, Harrisonburg, VA 22807. This project is a model of a hypothetical extraterrestrial biological attack on the planet earth. The model used is a modified Kermack-McKendrick S-I-R model for infectious diseases. The model itself tracks the progression of a zombie outbreak throughout the population of the Earth. It takes into account three primary factors: susceptible humans in the population, the number of zombies created in relation to the population, and the process of reanimation. Each equation also has several factors that are used to judge the spread of infection throughout the population: a birth rate that is consistent with the population, natural death, reanimation, and contact with an infected individual. A Zombie attack presented several challenges due to the fact that there is no known natural immunity or cure, and that the members of the population who die either naturally or by contact with a zombie, will reanimate and become a zombie. The method used to solve the S-I-R differential equations is the Parker-Sochacki method, developed by Dr. Edgar Parker and Dr. James S. Sochacki of James Madison University's Dept. of Mathematics and Statistics. The software programs Maple 13 and MatLab were used to model the progression of the attack through the population once the equations were set up. This was a group project between the presenters' Math 441 class at James Madison University.

# Biology (including Microbiology and Molecular Biology)

CORRELATION OF CHRONIC DISEASES WITH THE PRESENCE OF TROPHERYMA WHIPPLEI DNA IN SALIVA. Muhammed Faizan Casim & Lynn O. Lewis, Department of Biological Sciences, University of Mary Washington, Fredericksburg VA 22401. Whipple's disease is a rare systemic infection caused by the

bacterium Tropheryma whipplei. The disease is mainly known for its non-specific symptoms, including mal-absorption, diarrhea, and weight loss. T. whipplei is in the family Actinomycetes, which are environmental microbes usually found in soil and water, so a population with a poor diet and limited healthcare access could have a higher chance of having T. whipplei. In this study, we looked for the presence of T. whipplei DNA in human saliva and a correlation with any symptoms subjects might have. We tested two different populations; a population from a local free clinic with limited healthcare access and a poor diet and a population from the university with better healthcare access and a better diet available. We tested 95 subjects from the clinic and 60 students from the university. We collected saliva samples in sterile tubes and then extracted nucleic acid by using a Qiagen DNA MiniPrep extraction kit. The DNA was amplified through PCR by using Promega Master Mix with specific T. whipplei primers TW27F/TW182R. The medical history of the subjects was confidentially recorded for later use to make correlations. We found no positives in students or clinic populations therefore we were not able to make any correlation of chronic diseases with the presence of T. whipplei. Our study detected no difference in T. whipplei incidence between these two populations.

ENDOGENOUS REFERENCE GENE VALIDATION FOR QRT-PCR STUDIES ON HUMAN VISCERAL ADIPOSE TISSUE. Rohini Mehta<sup>1,2</sup>, Aybike Birerdinc<sup>1,2</sup>, Noreen Hossain<sup>2,3</sup>, Arian Afendi<sup>2,3</sup>, Vikas Chandhoke<sup>1,2</sup>, Zobair Younossi<sup>1,2,3</sup> & Ancha Baranova<sup>1,2</sup>, <sup>1</sup>Molecular and Microbiology Dept. and Center for the Study of Genomics in Liver Diseases, George Mason Univ., Fairfax, VA 22030. <sup>2</sup>Translational Research Institute, Inova Health System, Falls Church, VA 22042. 3Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA 22042. Real-time PCR (qRT-PCR) is the standard method for studying changes in relative gene expression in different tissues and experimental conditions. However, variations in amount of starting material, enzymatic efficiency and presence of inhibitors can lead to quantification errors; therefore the need for accurate data normalization is vital. Among several known strategies for data normalization, the use of reference genes as an internal control is the most common approach. Recent studies have shown that both obesity and presence of insulin resistance influence expression of commonly used reference genes in omental fat. In this study we validated expression stability of eight selected candidate reference genes using visceral adipose samples from obese (n=10) and lean (n=9) individuals. To determine the expression stability, RNA expression levels were measured in 19 visceral adipose tissue samples and cross-validated using three popular algorithms, GeNorm, NormFinder and BestKeeper. We recommend ACTB and RPII as stable reference genes most suitable for gene expression studies of human visceral adipose tissue.

MECHANISMS OF NEUROPEPTIDE AF-INDUCED ANOREXIA IN CHICKENS. Brandon A Newmyer<sup>1</sup>, Paul B Siegel<sup>2</sup>, and Mark A Cline<sup>1</sup>. <sup>1</sup>Department of Biology, Radford University, Radford, VA,24141. <sup>2</sup>Department of Animal and Poultry Science, Virginia Tech, Blacksburg, VA, 24060. Recently, we demonstrated the anorectic effect of neuropeptide AF (NPAF) when intracerebroventricularly (ICV) administered to

Cobb-500 chicks, which was associated with changes in hypothalamic chemistry. The purpose of the present study was to better elucidate the pathways to NPAF-induced anorexia and to test NPAF in models of hypo- and hyperphagia. In Exp 1-3, we selectively antagonized the mu, kappa, and delta subtypes of opioid receptors and coinjected NPAF in order to if NPAF'S anorectic effect was mediated through either of these subtypes; our results demonstrate that NPAF's anorectic effect is mediated through the mu and kappa but not delta subtypes of opioid receptors. In Exp 4-5, we cannulated both divisions of the paraventricular nucleus and administered microinjections of NPAF directly to these nuclei in order to determine which nucleus, if either, was the primary site of action in the NPAF pathway to anorexia. Our results demonstrated that NPAF did not affect food intake in chicks with cannulas targeting either nucleus, suggesting that neither region is the primary site of action in this pathway. In Exp 6-7, we ICV administered NPAF to chicks selected for low or high body weight and examined their food intake and behavior. We demonstrated that NPAF causes differential effects in these chicks and these effects are not associated with any behaviors competitive with appetite.

GASTRIN-RELEASING PEPTIDE CAUSES PRIMARY ANOREXIGENIC EFFECT IN CHICKENS. Collette R. Dougherty & Mark A. Cline, Radford Univ., Radford, VA 24012. The 27 residue gastrin-releasing peptide (GRP) is a secretagogue vital to digestion. We studied the effect of GRP on appetite. In Experiment 1, chicks that were injected intracerebroventricularly (i.c.v.) with GRP reduced both food and water intake in a dose-dependent manner. In Experiment 2, food-restricted chicks did not reduce water intake in response to i.c.v. GRP. Thus, the effect on water intake in Experiment 1 was prandial. GRP-treated chicks did not have reduced alimentary canal transit time for a gavaged red marker in Experiment 3, thus the effect in Experiment 1 may not be gut in origin. GRP-treated chicks had fewer feeding pecks. Behaviors including exploratory pecks, defecations, drinks, jumps and time spent standing, sitting, perching, and preening were not affected by GRP injection, indicating that the effect of GRP on appetite regulation is likely a primary effect. Thus, it is possible that GRP could be a sufficient appetite suppressant in humans.

DISPERSION AND ORIENTATION OF EGG CASES OF THE CHINESE PRAYING MANTIS ON WAX MYRTLE TREES. Heidi Etter & Robert K. Rose, Department of Biol. Sci., Old Dominion University, Norfolk, Virginia 23529-0266. The Chinese praying mantis, Tenodera aridifolia sinensis, was introduced to North America in 1896 near Philadelphia, Pa. A generalist insect predator inhabiting temperate successional old growth fields, this mantis is univoltine and can be iteroparous or semelparous depending on the region it inhabits. The study site for our project was a 7-year-old successional old field with volunteer wax myrtle trees located in Chesapeake, Va. One objective of the study was to determine if early laid egg cases hatch earlier than later laid egg cases. Preliminary results support this hypothesis. A second objective sought to learn if as the season progresses, egg cases are laid higher in trees and laid in a more southerly compass orientation. (An earlier study conducted

near our study site found the majority of egg cases to be oriented in a southerly direction and often high in trees. In late autumn, a southern orientation at heights increases the body temperature of the female mantis and thus her ability to convert food into eggs.) A third objective was to determine if the mean weight of the egg cases decreases throughout the season as food becomes more limited. Preliminary results support the latter study objectives.

THE CHANGE IN SMALL-SCALE SPATIAL DISTRIBUTION OF A SMALL MAMMAL COMMUNITY THROUGH OLDFIELD SUCCESSION. Jay P. Kiser & Robert K. Rose, Dept of Biol. Sci., Old Dominion University, Norfolk, VA 23529-0266. The distribution of a community of small mammals was evaluated during the succession of an oldfield habitat into a young pine forest, From 2002 through 2009 a capture-mark-release (CMR) study was conducted on a one-hectare grid at the Su Tract of Chesapeake Virginia. Over this time the small mammal community had variably consisted of cotton rats (Sigmodon hispidus), meadow voles (Microtus pennsylvanicus), marsh rice rats (Oryzomys palustris), eastern harvest mice (Reithrodontomys humulis), house mice (Mus musculus) and, towards the end, golden mice (Ochrotomys nuttalli). During the course of the CMR study, the change in succession was defined by the presence and density of the loblolly pine trees (Pinus taeda) found throughout the study area. Measurements of all the loblolly pines were taken in the winters of 2005 and 2008 and used to calculate the living basal areas throughout the grid. Associations were examined through regression analysis of the basal areas and captures of each small mammal species. House mice and meadow voles were found to be significantly negatively correlated with the living basal area, whereas rice rats showed no correlation. Cotton rats and harvest mice were significantly positively correlated with basal area up to a moderate level of pine growth and then negatively correlated when pines were taller.

GROWTH AND SURVIVAL OF VOLUNTEER LOBLOLLY PINE (*PINUS TAEDA*) TREES IN AN OLD FIELD IN EASTERN VIRGINIA. Robyn M. Nadolny & Robert K. Rose, Department of Biol. Sci., Old Dominion Univ., Norfolk VA 23529-0266. Old field succession to mixed hardwood forest is often prefaced by the invasion of open spaces by fast-growing loblolly pines (*Pinus taeda*). We examined growth rates, sources of mortality and demographics of volunteer loblolly pine trees within a 1.23 ha oldfield study grid in Chesapeake, Virginia. In the winter of 2005 we learned that 15.1% of 15,675 pine trees in a 5-year-old oldfield had been killed by girdling, and a further 50.0% partially girdled, by a high density of hispid cotton rats (*Sigmodon hispidus*). Three years later we examined sources of mortality after the rodent population had dropped to near zero. During the winter of 2008 the 7-year-old pine forest lost 138 trees (0.8%) to girdling but 23.0% (3,846 stems) to natural mortality, among 16,766 stems >0.8 m tall on a 1.23 ha grid. We measured the trees again in the winter of 2009-2010 to examine the rates of growth (basal area) from period to period, and to evaluate the effects of stem density on growth and survival. We conclude that

there was a drastic shift in mortality source between 2005 and 2008-10, and that high tree density predicts higher rates of mortality in all stages of succession.

INIHIBTION OF METHACILLIN RESISTANT STAPHLYCOCCUS AUREUS BY UNIQUELY SYNTHESIZED IMIDAZOLE ANTIMICROBIALS. Caitlin J. Hubbard, Randall D. Hubbard, & A. Garth McGibbon, Dept. Of Biology and Chemisty, Liberty University, Lynchburg VA. 24502. Substituted benzene rings containing a primary amine were selected. Diazitization of the amine was carried out using NaNO<sub>3</sub> at low pH and -10°C. This allowed for the formation of an unstable azynyl intermediate. Imidazole, an aromatic nitrogen containing, five membered ring, was subject to deprotonation at N1 at pH 14.5 using 50/50 base. Resonance created a nucleophile on the 2C, which in turn attacked the benzene azynyl group; this resonates to create an azo bond between the two ring systems. Azo coupling completion was visualized by color change due to change in absorbency properties; acidification of the solution led to protonation and precipitation of the product, which was repeatedly recrystalized to ensure purity. Compounds synthesized were (E)-(2-chlorophenyl)-(1H-imidazol-2-yl)diazene, (E)-(3chlorophenyl)-(1H-imidazol-2-yl)diazene, (E)-(4-chlorophenyl)-(1H-imidazol-2yl)diazene, 4-[(E)-1H-imidazol-2-ylazo]phenol, and (E)-1H-imidazol-2-yl-(4methoxyphenyl)diazene. Compounds were dissolved in 10% DMSO with 5% acetone. Kirby-Bauer disk diffusion technique was used to evaluate compound inhibition against community and clinically acquired MRSA strains. Compounds 2 & 5 were most effective for clinical MRSA strain, while compounds 1 & 5 were most effective for community MRSA strain. The addition and placement of large functional groups to the benzene ring increases the MRSA inhibitory properties of the imidazole derivatives.

THE ROLE OF A DEFENSIN PROTEIN IN ARBUSCULAR MYCORRHIZAL DEVELOPMENT. Barbara B. Kreutzer, Oscar R. Rocha & Rajendra P. Kandel, Dept. of Biol. and Phys. Sci., Marymount University, Arlington, VA 22207. Defensin proteins are found in many organisms and act as an efficient part of innate immune antifungal response. We hypothesized that some herbaceous plants express a brassica defensin protein which prevents symbiosis with mycorrhizal fungi. The defensin gene was found in nonhosts but not in hosts. SDS-PAGE indicates the gene was expressed in *Brassica rapa* exposed and not exposed to AM inoculum. Implications for AM development and plant and animal pathogenesis were discussed.

THE ISOLATION AND IDENTIFICATION OF A NEW CAENORHABDITIS SPECIES TO BE USED IN COMPARATIVE GENOMICS. Ann Wang, Erika Baardsen, & Theresa Grana. University of Mary Washington.

Nematodes are the most abundant multicellular organisms on the planet. To date, the most important nematode species to the scientific community is *Caenorhabditis* 

elegans. C. elegans is commonly used in developmental, neurobiological, and genetics research because it has a defined cell lineage, is easily maintained in a laboratory, and reproduces quickly. It was also the first multicellular organism to have its genome sequenced. Many researchers have benefited from comparative studies between C. elegans and C. briggsae. These species share many of the same characteristics, however, there are important morphological differences between the two nematodes. Researchers now agree that a more closely related species that exhibits a greater degree of morphological and molecular similarity will be isolated. The discovery of a new sister species would be beneficial to the C. elegans research community. Four nematode species have been isolated from Fredericksburg, Virginia. 4D imaging suggests that their internal structures differ from C. elegans. However, they also demonstrate some morphological and behavioral similarity to C. elegans. The 1A, 6A, 4D, and 2G species have been sequenced and aligned with the C. elegans 18S ribosomal sequence. These sequences differ significantly, leading us to conclude that these species are not sister species of C. elegans and would be of little use in comparative genomic studies for this project.

ANNOTATION OF THE TERMITE METATRANSCRIPTOME. Neerja Katiyar<sup>2</sup>, Natalie Fedorova<sup>1</sup>, Alan Lax<sup>1</sup> Gennady Denisov<sup>2</sup> & William C. Nierman<sup>3</sup>, George Mason University<sup>2</sup>, Fairfax, Virginia, 22030 and J. Craig Venter Institute<sup>1,2,3</sup>, Rockville, The sequencing of mRNA transcripts from the Formosan subterranean termite, Coptotermes formosanus, resulted in 131,637 Sanger reads and 6,942,682 Illumina reads. Sanger reads were assembled into 25,939 unigenes, which represent up to 60% of the species transcriptome. Phylogenetic analysis showed that over 50% of unigenes exhibited no sequence similarity to other proteins or PFAM and TIGRFAM domains and may, thus, represent termite-species specific genes or noncoding RNAs. Fifty percent of the other unigenes shared similarity with other insect genomes and 25% with Trichomonada, suggesting the presence of protozoan endosymbionts (parabasalids). Pathway analysis suggested that the majority of carbohydrate, glycan, and xenobiotic pathways in the Metabiome were contributed by endosymbiotic parabasalids. In addition, the CAZy system was used to classify enzymes involved in the carbohydrate metabolism. Expression levels of the 20% most abundant transcripts from one termite cast, Cf4, were captured using RNA-Seq technology. The most abundant unigenes included two Drosophila homologs: Tequila, which is essential for information processing in, and myofilin, which is required for filament assembly in all muscles.

CARDIAC STEM CELLS: MIGRATION ASSAY UTILIZING VEGF AND STATINS. Stacey Rickard & Kathryn E. Loesser-Casey, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA, 22401. Conventional treatments of heart disease do not result in myocardial restoration. However, recently discovered cardiac stem cells have been found to proliferate and differentiate into functional cardiomyocytes, giving researchers hope for use in treatment of heart disease. Understanding the factors that

influence the migration of these cardiac stem cells is paramount and therefore, my study examined the in vitro chemotaxic capabilities of 50 ng/ml VEGF and 0.1 µM rosuvastatin as well as the effect of age on cardiac stem cell migration. Cardiac stem cells were isolated from the hearts of newborn, 6-7 week old and 27 week old Sprague Dawley rats. Migration assays were performed on cells cultured from the newborn and 6-7 week old rats. Cell morphology indicated that both of the younger two age groups of rat cells appeared to be cardiac stem cells but the 27 week old rat cells appeared to be adipocytes. This indicates that the younger cardiac stem cells may be better suited for myocardial regeneration. The data from the migration assays produced no significant results when comparing the migration factors used, however, the newborn rat cells had significantly less migrated cells compared with the 6-7 week old rats. However, there was a second, unintentional variable. This study found that at the tested concentrations, VEGF and rosuvastatin did not induce cardiac stem cell migration. This study also supports the idea that younger cardiac stem cells are better suited to proliferate and differentiate into cardiomyocytes but age impacting the migration of cardiac stem cells cannot be concluded.

AGE-RELATED CHANGES IN STEM CELL MARKERS. Dana Hunt & Kathryn E. Loesser-Casey, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA, 22401 The use of stem cells to promote endogenous repair of cardiac tissue provides hope for millions of Americans suffering from cardiovascular disease. Adipose tissue may provide an easy, noncontroversial supply of stem cells. However, the origin of the fat, culturing conditions, and media formulations can influence the proliferation rate and differentiation capacity of these stem cells. Mesenchymal stem cells are reported to express CD105 and lack the hematopoietic lineage markers including CD34. However, studies have demonstrated that CD34 shows high levels of expression early in passage. This study was conducted to examine whether CD34 and CD105 expression varied due to location of fat and/or the number of days the cells were cultured. Brown fat was isolated from male laboratory mice from two locations, between the scapulae and in the groin. Isolated cells were grown on slides and CD34 and CD105 were localized using immunocytochemistry. The total number of positive cells were counted and a one-way ANOVA was performed.. CD105-positive cells were found to be statistically more abundant in adipose isolated from both between the scapulae and in the groin than CD34 labeled cells or control. Control slides (no antibody) did however, show some staining indicating that we need to repeat the experiments to reduce nonspecific binding of the antibodies. Our study supports the loss of CD34 markers with cell age and the presence of CD105-positive stem cells in adipose from several locations in the mouse.

Heat Shock Protein 60 Detection by Enzyme-Linked Immunosorbent Assay in House Sparrows (*Passer domesticus*). W. Humayon, A. Dolby, & D. O'Dell, Dept. of Biol., Univ. of Mary Washington., Fredericksburg, VA 22401. Stress is caused by everchanging environmental conditions that affect every organism. The stress response, which includes both release of stress hormones and production of heat shock proteins

(HSPs), protects animals from such biological challenges. Furthermore, not only are HSPs being applied to avian stress research on a limited basis, methods currently being employed by ornithologists to measure them do not allow them to be precisely quantified. These methods, based on colorimetric protein detection, only permit subjective comparisons to be made among samples. The objective of this project was to determine the efficacy of an alternative colorimetric HSP60 detection method that allows objective quantification, which would allow more meaningful analyses to be carried out of the factors that contribute to stress. Blood samples were collected from 16 House Sparrows at the University of Mary Washington campus during the spring of 2008 and 2009. Indirect and trap Enzyme-linked immunosorbent assays (ELISA) were used to measure HSP60 protein levels in them. Both methods detected HSP 60 proteins in the samples, but the trap ELISA was found to be more sensitive and showed less variability than the indirect ELISA. Funds were provided by Mrs. Thyra Valade Memorial Fund and UMW Undergraduate Research Fund. Thanks to V. Zimmermann, K. McAndrew & A. Dougherty for collecting samples.

BISPHENOL A INTERACTS WITH ESTROGEN RELATED RECEPTOR GAMMA TO REGULATE PRODUCTION OF C-FOS AND PS2 GENE PRODUCTS. Shannon Tucker. Department of Biology, University of Mary Washington, Fredericksburg, VA 22401. Bisphenol A (BPA) has been linked to breast carcinoma in humans for over 20 years, yet the mechanism by which BPA causes breast cancer has yet to be determined. We propose a novel mechanism by which bisphenol A acts through the estrogen related receptor  $\gamma$  (ERR $\gamma$ ), to upregulate potentially cancerous proto-oncogenes such as pS2 and c-fos. Our results show that blocking either the estrogen receptors (ER) or ERR $\gamma$ , (by use of fulvestrant and 4-hydroxytamoxifin, respectively) in human breast cells reduces the levels of c-fos protein and pS2 in cells exposed to carcinogenic levels of BPA, with the greatest reduction occurring in the ERR $\gamma$  blocked cells. Moreover, when both of these receptors are blocked, our results showed even lower amounts of c-fos (72.4% lower than control) and levels of pS2 proteins were undetectable. With further research, these results could finally explain the positive correlation between levels of BPA and breast cancer.

# **Biomedical and General Engineering**

LACK OF EFFECT ON CELL-MEDIATED IMMUNITY FOLLOWING IN VIVO EXPOSURE TO ELECTROSPUN POLYCAPROLACTONE. C.E. McLoughlin1, M.J. Smith2, G.L. Bowlin1, & K.L. White, Jr.2 1Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284, 2Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23284. Studies in our laboratory have focused on the effects on the immune system following in vivo exposure to electrospun polycaprolactone (EPCL). We are investigating the role of fiber diameter of EPCL, specifically comparing EPCL with average fiber diameter of 1.7um ("microfibrous") compared to 225nm ("nanofibrous").

The results presented demonstrate a lack of effect of EPCL on cell-mediated immunity, evaluated using the anti-CD3 T-cell proliferation and the mixed lymphocyte response assays following exposure to microfibrous EPCL. In addition both forms of EPCL have been evaluated in vivo with the delayed-type hypersensitivity (DTH) response. Neither microfibrous nor nanofibrous EPCL adversely affected the DTH response. Additional studies will include evaluation of both forms in not only young but also elderly animals.

APPLICATION OF HRV FREQUENCY DOMAIN METHOD ON RESPIRATORY RATE OF MECHANICALLY VENTILATED ADULTS. N.Y. Isti Arief, Paul A. Wetzel<sup>1</sup>, Mary Jo E. Grap, & Curtis N. Sessler Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. The Frequency domain method of heart rate variability (HRV) has been commonly used as an indicator of the physiological state of the cardiac and autonomic nervous system; a valuable tool for non-communicative, vulnerable population such as the mechanically ventilated. This study explores the relationship between respiratory sinus arrhythmia (RSA) and HRV by utilizing the frequency domain method on 10 intubated, sedated, mechanically ventilated adults. Correlation analysis was performed on power spectral density (PSD) estimates derived from heart and respiratory rate tachograms. Results showed modest overall correlation (R = 0.74, SD = 0.17, p < 0.0001), consistent with expectations for this population. This result supports the exploration for respiratory rate tachogram PSD as a potential tool for supplemental non-invasive autonomic nervous system indicator.

STRUCTURAL OPTIMIZATION FOR A NANO-FLUIDIC SYSTEM MIMICKING THE TRANSPORT THROUGH NUCLEAR PORE COMPLEX. Jae H. Lee & Ramana M. Pidaparti, Deptartment of Mechanical Engineering, Virginia Commonwealth University, Richmond VA 23284. A bio-inspired nano-fluidic system mimicking the nuclear pore complex (NPC) is investigated for fluidic transport by optimizing the geometry. In general, nuclear pore complex contains very distinct geometrical components to allow various macromolecules very effectively through the pore. In order to understand and design fluidic systems for drug delivery and other applications, this study explored the optimization of a central plug location of NPC for achieving the maximum velocity for fluidic transport. The simulation is repeated for the different positions and length to get the output velocity. The approach involves conducting fluid simulations with ANSYS software and optimizing the results using EXCEL software. Based on the results obtained, one configuration of the central plug location achieved maximum velocity through the modeled nano-fluidic system.

OCULOMOTOR CONTROL IN PATIENTS WITH PARKINSON'S DISEASE. George T. Gitchel M.S. Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. There have been few studies investigating the eye movement behavior of Parkinson's disease patients during fixation. This study objectively measured the eye movements of 36 patients with Parkinson's disease, and 20 age matched controls. Stimuli consisted of ten standardized text passages first organized by Miller and Coleman. In addition, subjects followed a randomly displaced step jump target motion. Pendular nystagmus was found

in all Parkinson's subjects, with an average frequency of 7.44 Hz. Saccadic peak velocity and duration along the main sequence were not statistically different from controls. A slower rate of reading was also noted in the Parkinson's group in terms of characters per minute, but with no more regressions than normal. Rate of square wave jerks was also found to be normal. This suggests that the hallmark feature of eye movements in Parkinson's disease is a pendular nystagmus during fixation, and all saccadic activity to be normal.

THE DESIGN AND VALIDATION OF A COMPUTATIONAL RIGID BODY MODEL OF THE ELBOW. E.M. Spratley, M.S., J.S. Wayne, Ph.D. Dept. of Biomedical Engineering, Virginia Commonwealth University. The use of computational modeling is an effective and inexpensive way to predict the response of complex systems to various perturbations. However, not until recently has this technology been used to predict the behavior of physiological systems, specifically the human skeletal system. To that end, a computational model of the human elbow joint was developed using computed topography (CT) scans of cadaveric donor tissue, as well as the commercially available software package SolidWorks<sup>™</sup>. The kinematic function of the joint model was then defined through 3D reconstructions of the osteoarticular surfaces The model was applied toward a cadaveric and various soft-tissue constraints. experiment performed by Fern et al that measured the significance of coronoid process fractures, lateral ulnar collateral ligament ruptures, and radial head resection in elbow joint resistance to varus displacement of the forearm. Kinematic simulations showed that the computational model was able to mimic the physiological movements of the joint throughout various ranges of motion including flexion/extension and pronation/supination. Quantitatively, the model was able to accurately reproduce the trends, as well as the magnitudes, of varus resistance observed in the cadaveric specimens. Additionally, magnitudes of ligament tension and joint contact force predicted by the model were able to further elucidate the complex soft-tissue and osseous contributions to varus elbow stability.

DEVELOPMENT OF A RIGID BODY COMPUTATIONAL MODEL FOR INVESTIGATION OF WRIST BIOMECHANICS. Benjamin J. Majors & Jennifer S. Wayne, Ph.D. Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. The wrist is one of the most complex joints in the human body because of the number of bony and soft tissue structures present to accomplish the activities of daily living. The goal of this study was to develop a computational model of the wrist joint complex where joint motion and behavior is dictated by highly accurate three dimensional articular contact, ligamentous constraints, muscle loads, and external perturbations. Validation of the model was achieved by comparing predicted results from the model to the results of a published cadaveric experiment that analyzed wrist function under effects of various surgical procedures. The results showed similar trends and magnitudes between the computational model and the cadaveric experiment. While some differences were seen, the model can still be used to predict overall biomechanical function of the wrist joint complex.

A COMPARATIVE ANALYSIS OF METHODS FOR BASELINE REMOVAL IN PRETERM INFANT RESPIRATION SIGNALS. Pallavi Ramnarain & Paul A. Wetzel, Ph.D. Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. Baseline drift removal is an critical step in preprocessing data for event detection applications. The goal of this work was to compare five different methods for basline drift removal in preterm infant respiration signals. These included a linear spline approximation, a cubic spline approximation, a recurrent neural network approach, a first derivative based approach and a second derivative based approach. Respiration was measured using a thermistor embedded in infant nasal cannula. All five methods were compared using the same event detection algorithm to evaluate their effectiveness at drift removal. The most effective method for this application was the second derivative method.

# **Botany**

THE FLORA OF VIRGINIA PROJECT: A 2009-2010 UPDATE. Marion B. Lobstein, Dept. of Biol., NVCC, Manassas, VA 22205. Virginia, for its landmass, has the most diversity of vascular plant species of any state in the United States. It had the first flora, the Flora Virginica in 1739, yet does not have a modern flora. The Virginia Academy of Science for over eighty years has supported efforts to produce a modern Flora of Virginia. In 2001 the Foundation of the Flora of Virginia, Inc, was formed in 2001 and in May 2002 received 501(c) 3 status. Progress continues to be made on the efforts to develop a Flora of Virginia including fund-raising and public outreach efforts. Work on the content of the Flora of Virginia including the nearly 300 of the core illustrations have been commissioned, completed, and funded by VAS funds. Grants from Robins Foundation and Old Dominion have been obtained for the Project during this past year. The Academy, including the Fellows, continues to provide essential support including financial for this Project. Other progress includes completion of treatments of the dichotomous keys of 190 of the 199 vascular plant families in Virginia and the first step in developing species and genus descriptions has been completed. The second step of herbarium work on descriptions is complete and the third and final step of species genus descriptions is 94% completed. The projected publication date is late 2012 or early 2013.

ANTIOXIDANT ANALYSIS OF SELECTED TEMPERATE SPICES. Erinda Stefi & Michael H. Renfroe, Dept. Biology, James Madison University, Harrisonburg VA 22807. There is a growing interest in natural antioxidants due to their major role in human health. Spices are main targets when searching for antioxidants. The addition of antioxidants in people's diets is very important in preventing degenerative diseases such as cancer, cardiovascular, neurological diseases and cataracts. The purpose of this research project was to determine the hydrophilic and lipophilic antioxidant content of seven Mediterranean spices: basil, oregano, sage, thyme, rosemary, marjoram and mint. The antioxidant content of each spice was directly related to the drop in ABTS absorbance upon the addition of the spice extract to the HAA and LAA reagents. A standard curve was established by measurements of known concentrations of trolox, a

well-characterized antioxidant that is soluble in both hydrophilic and lipophilic assays. For each spice, a statistical analysis of the variance and the comparison of sample means were performed using the analysis of variance, followed by the Dunnett's T3 post-hoc test (  $\leq 0.05$ ). For all the spices analyzed, higher HAA and total antioxidant activity (TAA=HAA+LAA) values were obtained from their ground form compared to their leaf form. The spice with the highest TAA of 36.25  $\mu$ mol TE/g fw was thyme, while the spice with the lowest TAA of 8.43  $\mu$ mol TE/g fw was sage leaves.

ANTIOXIDANT ANALYSIS OF SELECTED TROPICAL SPICES. Melanie M. Pommer & Michael H. Renfroe, Dept. of Biol., James Madison Univ., Harrisonburg VA 22807. Antioxidants are molecules which react to neutralize free radicals before they can damage biological molecules. The human body produces these naturally; however, the body's supply of antioxidants often is not sufficient to neutralize all free radicals. In this case, one may consider consuming foods or beverages that are rich in various antioxidants. Some common antioxidant-rich foods and beverages are fruits, vegetables, tea, coffee, red wine, soy products, herbs, and spices. The purpose of this research was to determine the antioxidant activity of seven ground tropical spices: mace, allspice, nutmeg, turmeric, cinnamon, cloves, and ginger. The ABTS/H<sub>2</sub>O<sub>2</sub>/HRP method was used to determine hydrophilic and lipophilic antioxidant content of each spice. The decrease in ABTS radical concentration due to antioxidant activity was monitored at 730 nm using a spectrophotometer. A standard curve was established using trolox. Reduction power of antioxidants extracted from each spice was reported as Trolox Equivalents (TE) in mol/g fresh weight. An analysis of variance and Dunnett's T3 test were conducted to determine significance of the difference of means (=0.05). Cloves, allspice, cinnamon, and mace were found to have the highest total antioxidant activity (TAA) ranging from 4.08 TE to 68.8 TE/g fw. Ginger, turmeric, and nutmeg had the lowest TAA ranging from 1.96 TE to 2.66 TE/g fw.

A TAXONOMIC REVISION OF THE ENDEMIC MEMBERS OF CORDIA L. (BORAGINACEAE) IN THE GALÁPAGOS ISLANDS. Julia K. Stutzman & Conley K. McMullen, Dept. of Biol., JMU, Harrisonburg VA 22807. The Galápagos Islands have long been an arena for biological diversity, scientific discovery, and more recently, conservation. Identification and documentation of the flora of the Galápagos can aid with conservation efforts. The purpose of the study reported here is to conduct a taxonomic revision of, and create an accurate taxonomic key for the four endemic members of the genus Cordia in this archipelago (C. revoluta Hook. f., C. leucophlyctis Hook. f., C. anderssonii (Kuntze) Gurke, C. scouleri Hook. f.). Taxonomic uncertainty among the species has resulted in the inability of these species to be evaluated for conservation status by the International Union for Conservation of Nature. taxonomic study of these Cordia species will determine how they are to be identified in the field. A related GIS analysis of the population locations of each species will assess species distribution on the islands. Proper identification and distribution assessment of the endemic Cordia populations will allow for a determination of the conservation status of each species.

OF FOLIAR GLANDS OF STIZOPHYLLUM RIPARIUM STRUCTURE (BIGNONIACEAE). M. J. Drake & W. John Hayden, Dept. of Biol., UR, Richmond, VA 23173. Stizophyllum riparium, is a liana widely distributed in the neotropics characterized by foliar glands associated with pellucid-punctae in the leaves. We investigated structure of these glands via light and scanning electron microscopy using specimens obtained from the Kaxil Kiuic reserve located in Yucatan, Mexico. The glands arise from protoderm, are located in small depressions on both leaf surfaces, and consist of three or four basal cells that support a disk-like gland body with upturned edges. Cells of the gland body radiate from the central basal cells to the upturned gland margin; these cells are elongated parallel to the underlying epidermis. Cuticle separates from the outer periclinal walls of the gland body consistent with a merocrine-eccrine mode of secretion. Mesophyll immediately adjacent to foliar glands remains undifferentiated (no distinct palisade and spongy layers) and lack chloroplasts, rendering gland-associated leaf regions translucent. Similar, but non-pellucid, glands are known in the Asian genus Incarvillea; however, most foliar glands in the family are characterized by a palisade-like secretory layer supported by either a single, grossly enlarged, basal cell, or numerous small basal cells. Foliar glands in Bignoniaceae can be interpreted as homologous with glandular trichomes.

SELECTIVE FORAGING OF DIATOMS BY THE MARSH PERIWINKLE (LITTORINA IRRORATA) IN THE CHESAPEAKE BAY ECOSYSTEM. Charlotte L. Clark & Harold G. Marshall, Dept. Biol. Sci., ODU, Norfolk, VA 23529-0266. The marsh periwinkle is a common snail in tidal mud flats of Virginia's estuarine marshes of the Chesapeake Bay. It is described as a grazer of detritus and algae as it moves across the surface sediment of these inter-tidal regions. Common components of this sediment are diatoms, both of sediment and plankton origin. The objective of this study was to determine to what extent these snails utilize diatoms as a food source. Replicate samples of snails (large and small) and sediment (ca. 30 g) were taken monthly (January to April 2010) in the exposed intertidal mud flat of the Lafayette River, Norfolk. The snails were brought back to the laboratory where their fecal pellets were then collected. Diatom frustules were counted in both the fecal pellets and the sediment. There were 35 diatom taxa identified in this process. Diatoms were considered living that contained a recognizable protoplast; those lacking a protoplast as non-living. The results indicated no significant difference in the presence of empty frustules between the sediment and fecal pellets, or between the size of the snail and the sediment. The results indicated Littorina irrorata did not efficiently utilize living diatoms as a food source. The snail's major food source is more likely to be soft bodied algae and detrital material than these diatoms.

INTEGRATING ECOLOGY AND GENETICS TO UNDERSTAND ADAPTATION TO NOVEL ENVIRONMENTS. Carrie A. Wu, Dept. of Biol., UR, Richmond, VA 23233. Specialization to local environmental conditions can generate the adaptive population differentiation that is required for ecological speciation. A particularly dramatic example of ecological specialization involves alpine endemics, which have evolved characteristic suites of adaptations, such as strikingly prostrate, cushion-like

growth forms, in response to similar selective environments. We are investigating the ecological and genomic basis of this common ecological transition to alpine environments using the mountain monkeyflower, Mimulus tilingii. In contrast to the widely distributed M. guttatus, M. tilingii populations are confined to subalpine and alpine habitats throughout western North America, in high altitude "habitat islands" that provide a unique set of replicate evolutionary experiments in trait divergence to common ecological influences. Despite the close geographic proximity of the progenitor species M. guttatus, we detected high levels of genetic and phenotypic divergence between the two species. Using nuclear microsatellites and chloroplast sequence data, we demonstrate that M. tilingii is indeed genetically distinct from M. guttatus, and actually comprises its own geographically structured species complex. Common garden studies indicate that the two species are primarily divergent in vegetative, rather than floral traits, consistent with patterns of reduced stature often observed in alpine plants. In addition, greater morphological than neutral genetic differentiation between the species suggests a major role for natural selection in maintaining the distinct, compact morphology of M. tilingii.

INTRODUCTION TO THE CAPE FLORISTIC REGION. W. John Hayden, & M. J. Drake, Dept. of Biol., UR, Richmond, VA, 23173. Floristic regions (kingdoms) can be defined on the basis of endemic taxa at relatively high rank (families, subfamilies, tribes). Most floristic regions coincide with major continental land masses and their biotic uniqueness can be readily explained as a consequence of isolation during past episodes of plate tectonic movement. In contrast, the miniscule Cape floristic region is manifestly distinct floristically despite having a tectonic history common with the African continent. Recent floristic studies recognize 8920 species of flowering plants, 69% of which are endemic to the region; the Cape region occupies a mere 0.5% of the land area of Africa but holds 20% of the continent's plant diversity. Endemic families include: Geissolomataceae, Grubbiaceae, Penaeaceae, Roridulaceae, and Stilbaceae (s.s.). Families with more than 10 genera endemic to the Cape region include: Asteraceae, Aizoaceae, Ericaceae, Fabaceae, Rutaceae, Proteaceae, Orchidaceae, Iridaceae, and Restionaceae. Several vegetation communities exist, but fynbos, a sclerophyllous shrub land adapted to fire and acidic, nutrient-poor soils dominates the region. Many plants from the Cape region have entered the horticulture trade and are now widespread in cultivation. On the other hand, the Cape region is considered a global biodiversity hotspot, with many pressing conservation needs. Overall, the Cape flora shows significant affinities to the flora of Africa and floras of the Southern Hemisphere (Gondwana).

FLOWER AND INFLORESCENCE STRUCTURE IN DORSTENIA (MORACEAE): AN UNDERGRADUATE DISCOVERY SCIENCE PROJECT. W. John Hayden, Dept. of Biol., UR, Richmond, VA, 23173. Flowers of Dorstenia are small, unisexual, structurally reduced, and densely aggregated into capitula; as such, they test well students' basic concepts of floral morphology. Student teams were provided with inflorescences from three species of Dorstenia from which they prepared paraffinembedded sections for study with light microscopy (LM); students also studied

inflorescence surfaces via scanning electron microscopy (SEM). LM and SEM images were pooled with macrophotographs and line drawings from the literature to create an image bank. In class, each student then created, from scratch, a short PowerPoint presentation designed to analyze/interpret flower and inflorescence structure, drawing as needed from the image pool. At first students balked at the relatively open-ended and unstructured nature of the assignment, but soon began to engage with the challenge of making sense of the flowers and the different ways that the same structure can appear when rendered by different imaging techniques. Students discovered for themselves the peculiar flowers of *Dorstenia*, thus broadening their understanding of the fundamentals of flower structure and floral diversity.

# Chemistry

IS TiF2 REALLY LINEAR? T.C. DeVore, Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807. The molecular geometry of the first row transition metal difluorides has been of interest to experimentalists and theorists for over 40 years. Recently, Wilson et al. concluded that the was no evidence that any of these compounds were non-linear. Density functional theory (DFT-B3LYP with a 3-611G++(3df,3pd) basis set) has been used to determine the molecular geometry and the vibrational frequencies for TiF2 and Ti2F6. These calculations indicated that the  ${}^3B_2$  ground state of TiF2 was non-linear with a bond angle of  $\sim 139.2^\circ$ . The bond length was 180.6 pm. The  ${}^3B_{2u}$  ground state of Ti2F6 has D2h symmetry with terminal and bridging bond lengths of 178.3 and 180.3 pm respectively. The terminal F-Ti-F bond angle is  $125.1^\circ$ . The IR active Ti-F stretching frequencies are 728.19 and 625.13 cm $^{-1}$  for TiF2 and 755.9, 681.0 and 513.0 cm $^{-1}$  for Ti2F6. The calculations indicate that in contrast to the recent conclusions reached by Wilson et al., the ground state of TiF2 is non-linear.

THE SYNTHESIS OF NITROGEN-AND SULFUR-CONTAINING HETEROCYCLES FROM CYCLO-PROPANOL FRAGMENTATION. Georgia T. Stoyanov, Kelly L. George & Kevin P.C. Minbiole, Deptment of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807. The prevalence of heterocycles as the backbone of common pharmaceutical entities has created a demand for simple reactions to prepare them. Our research aimed to create six-and seven-membered heterocycles containing both a carbonyl group and either sulfur or nitrogen in the ring. This is modeled after a cyclopropanol fragmentation approach to the formation of oxepanes developed previously in our group. Our current endeavor is to synthesize nitrogenous heterocycles, specifically piperidines and azepines, as well as sulfur-containing thiepanes. The nitrogenous approach begins with suitably protected α-or β-amino acid ethyl esters which were transformed by cyclopropanols via the Kulinkovich reaction. The resulting α-or β-amino cyclopropanols were then reacted with various aldehydes to form an aminal. Subsequently, various Lewis acids were investigated to promote the rearrangement of the aminal into the piperidine or azepine. Analogously, a seven-membered sulfur-containing heterocycle was formed, albeit in low yields.

ANTHOCYANIN AND ALUMINUM CONTENT OF RED AND BLUE SEPALS FROM SELECTED HYDRANGEA MACROPHYLLA CULTIVARS. Schreiber, Samantha E. Wade, Kelly M. Mayhew, Andrew H. Jones & Judith B. Cain, Department of Chemistry, Virginia Military Institute, Lexington VA 24450. The primary pigment in sepals (modified leaves comprising the inflorescences) of hydrangea is an anthocyanin, delphinidin-3-glucoside. In red sepals, this anthocyanin occurs as its red flavylium cation. In acidic soil, aluminum as Al<sup>3+</sup> is incorporated through the roots into the shrub and is transported to the sepals where A13+ forms a complex with the blue quinoidal base anion of this anthocyanin. Analyses of the red and blue sepals of numerous hydrangea cultivars show that whereas red and blue sepals of the same cultivar have the same anthocyanin content, individual cultivars can be classified by their unique anthocyanin contents. For example, the popular remonant or cold-hardy cultivars have 80-120 µg anthocyanin per g fresh sepal, while the most vibrantly colored cultivars have over 300 µg anthocyanin per g fresh sepal. The anthocyanin content of a particular cultivar is typically proportional to its perceived intensity of coloration. Bluing of the sepals on the average require about a 10-fold molar excess of aluminum over anthocyanin, meaning that the threshold aluminum content for bluing is also cultivar dependent. Experiments have also shown that greater aluminum contents than the threshold for that cultivar do not result in bluer sepals, in agreement with a chemical model for bluing.

CHEMICAL CONTROL OF PAPERWHITE (NARCISSUS TANZETTA ZIVA) GROWTH AND FLOWERING. Timothy V. Johnson & Henry D. Schreiber, Department of Chemistry, Virginia Military Institute, Lexington VA 24450. Paperwhites, Narcissus tanzetta Ziva, are plants that are commonly grown from bulbs for winter blooming indoors. They are known for their stunning white flowers, but these flowers are often perched on top of a much too-tall stem that seems out of proportion to the flowers. In a previous study, paperwhite bulbs were grown in alcoholic solutions (optimal 5 vol% ethanol) instead of water, resulting in shorter stems to remedy the plant's "floppiness" without sacrificing floral quality. This study expanded the prior work by testing a wider variety of alcohols and other common laboratory and household chemicals. Growing paperwhites in a 5 vol% solution of ethanol in water indeed stunted the stems of the plants, but also lessened the number of flowers, in contradiction to the previous study. 5 vol% solutions of ethanol, methanol, isopropanol, ethylene glycol, glycerol, and acetone in water were all effective at stunting the stem growth by 33-50%, but the number of flowers also tended to be about 33% less than the control (water). The size of the blooms as well as the bloom period remained unaffected by the chemical additives. The chemical additives appeared to stunt but not kill the paperwhites, for which the additives acted as a mild toxin. Thus, although alcoholic solutions are effective in producing dwarf paperwhites, the previous conclusion that floral quality remains unaffected is misleading.

SUBUNIT INTERACTION OF THE CAP METHYTRANSFERASE. Joolan Saroor, Jessica N. Skeeter, Jeanhee Chung & <u>Thomas O. Sitz</u>, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The 5'-end of eukaryotic mRNA is capped and

methylated in the N-7-position of the guanine base generating a fully functional cap structure. If the cap is not methylated at this position, the mRNA is not translated, i.e. this methylation is essential for gene expression. The enzyme that methylates the N-7 position, Guanine-7-methyltransferase, has been expressed as a His-tag protein in E. coli. The addition of histidines at the N-terminus allows the enzyme to be purified on a Nickel column. The full length enzyme, 476 amino acids long, and the deletion mutation, 120 amino acids removed from the N-terminus, are about 80% pure after the nickel column. To further purify the enzymes, a positively charged ion-exchange column (Mono Q-Sepharose) was used resulting in greater than 95% purity. This purified guanine-7-methyltransferase (full length and deletion mutation enzyme) was then applied to a FPLC-Superose 12 gel exclusion column and two major peaks of protein were observed for both the full length and the deletion mutation enzyme which corresponded to about 90% homodimer and 10% monomer for each respectively. The purified enzymes were also analyzed by blue-native polyacrylamide gel electrophoresis and the deletion of 120 amino acids had no affect on the subunit interaction, i.e. about 90% dimer. The enyme samples were then subjected to cross-linking with 1-Ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Both the full length and deletion mutation were not cross-linked, suggesting that dimers were not formed by charge interaction (electrostatic interaction).

POLYCEPHALIC (MULTI-HEADED) CATIONIC AMPHIPHILES AS NOVEL Robby Davis<sup>1</sup>, Christian SURFACTANTS AND ANTIMICROBIAL AGENTS. Schwantes<sup>1</sup>, Devon Flaherty<sup>2</sup>, Kevin Caran<sup>1</sup>, Kevin Minbiole<sup>1</sup> & Kyle Seifert<sup>2</sup>, <sup>1</sup>Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807 and <sup>2</sup>Department of Biology, James Madison University, Harrisonburg VA 22807. We recently reported the synthesis and colloidal study of a novel series of biscationic bicephalic amphiphiles, each with two charged head groups and a single nonpolar tail connected via an arene core. Initial biological testing showed that six of seven amphiphiles tested were antibacterial and/or antifungal; several inhibited bacteria more effectively than ampicillin. We aim to build on this preliminary data and investigate the structure dependence and mechanism of antimicrobial activity of a wide range of cationic multiheaded amphiphiles. We will incorporate different substitution patterns as well as vary the cationic and hydrophobic groups to access a diversity of related amphiphilic structures. Colloidal characteristics of these surfactants will be assessed. We plan to determine the MIC and mechanism of action of each synthesized compound. Then, correlation of monomeric structure, aggregation tendencies, and mechanism of action will be examined.

AMPHIBIAN CHEMICAL DEFENSE: IDENTIFICATION AND APPLICATION OF ANTIFUNGAL METABOLITES FROM JANTHINOBACTERIUM LIVIDUM AND PEDOBACTER CRYOCONITIS. <u>Jacob Smith</u> & Kevin P. C. Minbiole, Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807. To develop a probiotic antifungal treatment against the deadly fungus <u>Batrachochytrium dendrobatidis</u>, anti-fungal metabolites from bacteria on the amphibians' skin were identified. As a preliminary step, metabolites from <u>Pedobacter</u>

cryoconitis separated through HPLC are currently being tested against B.d. A previous study of the mountain yellow-legged frog (Rana muscosa), infected with B.d., provides a model for the probiotic treatment of amphibian populations with Janthinobacterium lividum. A study is underway to measure the effects of Janthinobacterium lividum against Chytridiomycosis on the extirpated Panamanian golden frog (Atelopus zeteki). A soil extraction protocol to detect violacein, currently under development, will allow for the identification of J. lividum in soil samples. If successful, this could simplify the transition from the lab to the wild.

THREE ARABIDOPSIS TALIANA MYO-INOSITOL 1-PHOSPHATE SYNTHASE GENES ENCODE BIOCHEMICALLY SIMILAR ENZYMES. Xinyi Huang, Marcy Hernick & Glenda E. Gillaspy,

Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. Inositol L-myo-inositol 1-phosphate synthase (MIPS; EC 5.5.1.4) catalyzes the conversion of D-glucose 6phosphate to 1L-myo-inositol 1-phosphate. The expression pattern and metabolite function of three MIPS genes from Arabidopsis thaliana have been characterized. In order to prove that these proteins were similar enzymes with similar catalyzing ability, MIPS1, MIPS2 and MIPS3 ORFs were cloned into vector pDEST17 and induced in E. coli BL21(DE3) strain. The N-terminal his-tagged MIPS proteins were purified by Ni-IMAC (Fig.1). The yield of MIPS proteins were among 4 to 20 mg/L culture. catalytic activity of MIPS proteins was measured under steady-state conditions, by coupling reaction with excessive amount of myo-Inositol monophosphatase (IMP, EC 3.1.3.25) at 30°C. The kinetic parameters  $(k_{cat}, K_{M}, k_{cat}/K_{M})$  were obtained by fitting the Michaelis-Menten equation to the initial linear velocities measured at the various substrate concentrations. The kinetic properties of MIPS1, MIPS2 and MIPS3 were not significantly different among each other (≥ 2 fold) Thus, we concluded that MIPS proteins were similar enzymes in plants, and the different impact on growth and cell death was due to the developmentally and spatially regulation of MIPS genes expression.

EXPRESSION AND PURIFICATION OF Rv0323c, A HYPOTHETICAL MYCOBACTERIAL PROTEIN. J. Boggia & M. Hernick, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. Mycothiol is the primary reducing agent used by mycobacteria to prevent against oxidative damage. Consequently, enzymes involved in mycothiol biosynthesis are targets for antibiotic development. One of the key steps in this pathway is the hydrolysis of GlcNAc-Ins to form GlcNH<sub>2</sub>-Ins and acetate. Under normal conditions, this reaction is catalyzed by the enzyme MshB. However, the MshB knockout is capable of producing some, albeit decreased, mycothiol indicating that there are one or more redundant enzymes. Based on sequence alignment data, there are two potential enzymes that may fulfill this function, mycothiol-conjugate amidase (MCA) and the hypothetical protein Rv0323c. We have cloned the genes for both of these enzymes into vectors that allow for the recombinant expression of these proteins in E. coli. We have been able to express and purify the protein encoded by the Rv0323c gene. Current efforts are focused on characterization of the Rv0323c function, and determination of whether it possesses GlcNAc-Ins deacetylase activity.

SOLUTION-PHASE SYNTHESIS OF CuPt BIMETALLIC CATALYSTS AND THEIR APPLICATIONS IN CO OXIDATION. Q. Liu¹, D. W. Goodman², J. D. Batteas² & R. E. Schaak³¹Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807, ²Department of Chemistry, Texas A&M University, College Station, TX 770842 and ³Department of Chemistry and Materials Research Institute, The Pennsylvania State University, University Park, PA 16802. A mixture of CuPt nanospheres and nanorods was directly synthesized in liquid phase. The characterization of Transmission electron microscopy (TEM) and X-ray diffraction (XRD) demonstrated that the atomic ratios of Cu/Pt in both spheres and rods are very close to 1. Moreover, the length of the nanorods can be tuned by changing reaction conditions in the range of 10 to 55 nm and a possible formation mechanism for these nanorods was hypothesized. To investigate the catalytic activities of resulted CuPt nanoparticles, the nanospheres and nanorods were separately deposited on Al<sub>2</sub>O<sub>3</sub> and then used as catalysts probing the light-off temperature for oxidation of CO to CO<sub>2</sub> in a closed system.

METHOD DEVELOPMENT FOR ELEMENTAL ANALYSIS OF FOLIAR BLUEBERRY (VACCINIUM CORYBOSUM L.) SAMPLES. Allison E. Glick, Denay M. Fuglie, Braydon P. Hoover & Roman J. Miller, Dept. of Chem., Eastern Mennonite Univ., Harrisonburg, VA 22802. Nutrient levels in blueberry leaves from established Blueray, Coville, and Jersey cultivars were analyzed by flame atomic absorption spectroscopy (FAAS) in a method development to use in research for a model organic blueberry farm. Foliar samples were oven-dried or oven-dried and dry-ashed at 450° C. then digested with 6 M HCl or concentrated HNO<sub>3</sub>, and compared for higher extraction of the elements Fe, Zn, Mn, Cu, Ca, and Mg. There were no differences in which acid is used to digest nor whether the sample is ashed. However, the blueberry cultivars did differ in elemental concentrations and cannot be grouped together for nutrient evaluation. Older leaves also had different elemental concentrations as measured by FAAS than younger leaves gathered from the same bush. FAAS could also detect difference between a healthy bush and one recovering from stress. Using oven-dried leaf samples digested with 6 M HCl is the method of choice to measure the nutrients Fe, Zn, Mn, Cu, Ca, and Mg. (Research supported in part by USDA Specialty Crop Grant # 2008-427, Commonwealth of Virginia, Department of Agriculture and Consumer Services.)

SPECIFIC ION EFFECTS ON PROTEIN AGGREGATION. Yanjie Zhang<sup>1\*</sup>, Branden Deyerle<sup>1</sup>, Justin Hagerman<sup>1</sup>, Paul S. Cremer<sup>2</sup>, <sup>1</sup>Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807 and <sup>2</sup>Department of Chemistry, Texas A&M University, College Station, TX 77843. Specific ion effect was first noted by a protein chemist, Franz Hofmeister, over a hundred years ago that ions showed varying abilities to precipitate protein molecules out of solution. This effect is known as a recurring trend in a variety of physical and biochemical processes such as protein stability, enzyme activity, and colloidal assembly. Despite its generality, the understanding about the mechanism of the Hofmeister effect on molecular level is far from complete. In this presentation, interactions between ions and

protein molecules were investigated in a temperature gradient microfluidic setup and the mechanism of the Hofmeister effect was elucidated.

BIOMINERALIZATION TEMPLATED BY AMINO ACID-BASED CHIRAL MOLECULES. <u>Justin Hagerman</u>, Branden Deyerle, Yanjie Zhang, Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807. Biomineralization is an extremely widespread phenomenon in the biological world. Examples of biominerals include silicates in algae and diatoms, carbonates in invertebrates, and calcium phosphates and carbonates in vertebrates. The functions of biominerals range from magnetic sensing to structural support. Herein, amino acid-based chiral molecules were employed as model systems to direct the growth of biominerals. The role of chiral molecules and the mechanism for the nucleation and crystallization of biominerals will be discussed.

HOFMEISTER EFFECTS ON THE PHASE BEHAVIORS OF THERMAL RESPONSIVE POLYMERS. Branden Deyerle, Justin Hagerman, Yanjie Zhang, Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807, Over the last 120 years, a wide variety of phenomena from protein folding and enzymatic activity to colloidal assembly and protein crystallization have been shown to follow the Hofmeister series. Despite its ubiquity, a molecular level understanding of the Hofmeister series is still lacking. Herein, we will employ a model system, phase transitions of thermoresponsive triblock copolymers, to investigate the mechanism of the Hofmeister series on the molecular level. The phase transition temperatures of these triblock copolymers were measured in the presence of Hofmeister anions and the data were correlated with the properties of anions.

EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO IDENTIFY SELECTIVE INHIBITORS OF CASEIN KINASE 1 FROM TRYPANOSOMA CRUZI. Sahil Khanna, Pablo Sobrado & David Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24060. Trypanosoma cruzi casein kinase 1 (TcCK1) is a multifunctional Ser/Thr protein kinase that catalyzes the phosphorylation of key proteins in regulation pathways. Its function in cell growth makes TcCK1 a potential drug target to prevent the growth of T. cruzi. Infection by T. cruzi parasite results in Chagas disease, known to cause sudden cardiac failure. Human casein kinase 1 delta (hCK1δ) is important to proper physiological function of human cells, therefore the drugs developed need to be selective towards TcCK1 over hCK1δ. Computational docking experiments of 14 potential inhibitors to both TcCK1 and human casein kinase 1 delta (hCK1δ) were performed using Dock6. Analysis of the ATP binding pocket of TcCK1 and hCK1δ showed exploitable structural differences, particularly spacing between residues E49/D149 in TcCK1 and E50/D149 in hCK18. The resulting grid scores have indicated 5 inhibitors for in vitro study, (R)-DRF053, Purvalanol B, CKI-7, PF670462, and D4476. TcCK1 was cloned into Top-10 E. coli cells and co-expressed along side chaperone protein complexes DnaK-DnaJ-grpE and GroEL-GroES. TcCK1 was partially purified utilizing an Immobilized Metal Affinity Columns (IMAC). A γ-<sup>32</sup>P-ATP assay was used to determine a Km value of 2.3±0.72 mg/mL for α-casein as

a substrate. A method to obtain active enzyme was formed, and can be used for further inhibition studies. Ki values for selective inhibitors determined by the *in silico* study can be obtained.

# **Computer Science**

USING SECOND LIFE FOR COMPUTER SCIENCE EDUCATION. Robert A. Willis Jr. Department of Computer Science, Hampton University. Over the past few years, I have noticed that our students are reluctant to approach learning computer science in the traditional ways. Computer science requires beginning students to learn the concepts of computer science and the art of programming. While disparate, both of these facets require a good deal of study using texts and practice. Second Life is used to implement a number of innovative interactive tutorials tailored for this generation of students. Furthermore, the environment is conducive for instruction in a number of other areas in computer science (and other disciplines). Second Life is a three dimensional virtual world. It is a social environment that allows people to "live" much as they do in real life. People (represented as avatars) can purchase land, build houses, work, play, and participate in many other activities. It is an ideal environment to reach all levels of students.

INTERACTIVE PARAPHRASE TRAINING: THE DEVELOPMENT AND TESTING OF AN ISTART MODULE. Chutima Boonthum. Department of Computer Science, Hampton University Hampton University. Comprehension of science texts is challenging, particularly when the reader lacks the skills or knowledge necessary to fill in conceptual gaps in the text content. The iSTART system was developed to help readers learn and practice reading strategies to improve their ability to comprehend challenging text. This study describes a new iSTART module recently developed and tested, called Interactive Paraphrasing (IP), in which students are interactively and adaptively taught how to paraphrase sentences. We compared the effects of iSTART to iSTART with IP (IP-iSTART) with high school students on their strategy use and ability to comprehend text. IP-iSTART increased skilled readers' self-explanation quality, improved their ability to answer online comprehension questions, and increased their use of paraphrases after training. Less skilled readers benefited most in self-explanation quality from the original version of iSTART. Results are discussed in terms of tailoring reading strategy training to the needs of the reader.

GENERATION Y AND COMPUTER LITERACY/EDUCATION. Angela Hayden. Department of Computer Science, Hampton University Hampton University. The generation of Americans born between 1977 and 1994 are affectionately known as Generation Y. They hold to similar values of their parents, but will challenge authority and the information given them in any setting. They possess a variety of skills including computer skills, making them the most computer literate of all generations prior to them. They can be stimulated through a variety of means, most of which are visual and audio. They also appreciate having fun more than just learning facts. Strategies for both study and pedagogy offered as suggested means to help students learn have not changed

much in recent years and can still be used for those entering college over the next two or three years. One such strategy includes visual/auditory where students are asked to read aloud, record and play back definitions to terms, or visualize certain tasks. At HU, we offer students in our CSC 120, Intro to Computer Literacy course a method that requires them to do much more than just passively sit in class and take notes. This method, where students learn computer applications using hands-on activities, is not without its problems and challenges, but overall most students do extremely well and some have express not only satisfaction with the course, but acknowledge that learning has occurred.

### Education

THE ART LOVER'S PROBLEM. W. Michael Gentry, Department of Mathematics, Mary Baldwin College, Staunton, VA. An application of algebra, without the use of calculus, to solve *The Art Lover's Problem*: How far from a Pablo Picasso portrait should an art lover stand in order to obtain the best possible view? Encourages first-year college students to think actively; helps them understand how a scientist sees or interprets the physical world. Patient problem-solving and algebraic skill are both necessary. Basic skills in algebra precede a deeper more theoretical understanding. Student responses indicate that although conceptual understanding should come first, it remains shallow in nature, unless and until some facility in algebra is developed.

STORMWATER MANAGEMENT AT THE SCIENCE MUSEUM. Lindsay M. Walker<sup>1</sup> & Eugene G. Maurakis<sup>2</sup>, <sup>1</sup>Randolph-Macon College, Ashland, VA and <sup>2</sup>Science Museum of Virginia, Richmond, VA. Stormwater runoff has been a growing problem for watersheds throughout the United States. As stormwater flows across paved surfaces, such as roads, parking lots, and roofs, it picks up trash, fertilizers, pesticides, PCB's, and other pollutants which end up in larger bodies of water such as the Chesapeake Bay. To improve the quality of water for local waterways such as the James River and the Chesapeake Bay, the Science Museum is collaborating with a variety of groups to implement stormwater management technologies; porous pavement, rainwater harvesting systems, green roof, BayScapes gardens, bioretention areas, and tree box filters. The Museum is creating exhibits and demonstrations which correlate to the Virginia Standards of Learning so the students can learn about stormwater management technologies. Funded by the National Fish & Wildlife Foundation.

CONNECTING GRADUATE RESEARCH TO UNDERGRADUATE AND SECONDARY SCIENCE EDUCATION. Lisa S. Webb<sup>1</sup>, Roberto A. Flores<sup>2</sup>, Geoffrey C. Klein<sup>1</sup>, Michael D. Meyer<sup>1</sup> & Gary J. Whiting<sup>1</sup>, <sup>1</sup>Department of Biology, Chemistry and Environmental Science and <sup>2</sup>Physics, Computer Science and Engineering, Christopher Newport University, Newport News, VA. CNU's NSF funded GK-12 project, Linking Urban Water Quality with Science Education in the Chesapeake Watershed, involves placing graduate Environmental Science and Computer Science

students in a 9<sup>th</sup> grade Earth Science classroom where they will utilize an interdisciplinary, guided-inquiry based approach to address an environmental question (i.e. what is the health of the local watershed and what events and actions influence it?). The project is interdisciplinary, integrating environmental science, basic and applied biology (ecology and entomology), chemistry and computer science, and includes a combination of classroom, laboratory and field experiences. In order to extend the project's connection to include undergraduate science education, we field-tested the labs and activities in an introductory biology laboratory for non-science majors. We describe the scope of the activities and discuss the success of utilizing this approach to connect graduate level research to undergraduate and secondary science education. The authors would like to acknowledge the generous financial support of the National Science Foundation (GK-12 Program, award # 0841295).

RESTORATION OF DEGRADED WETLANDS IN AN URBAN SETTING: A COMMUNITY PARTNERSHIP. M. T. Muller<sup>1</sup>, M. S. Semcheski<sup>1</sup>, T. A. Egerton<sup>1</sup>, C. L. Clark<sup>1</sup> & K. DuBois<sup>2</sup>, <sup>1</sup>Department of Biology, Old Dominion University, <sup>2</sup>City of Norfolk. Considering the drastic loss of tidal wetlands in the Chesapeake Bay Watershed over the past century, a sense of environmental stewardship has began to percolate. Urban environments provide a distinctive suite of challenges for wetland restoration that requires cooperation from a number of city officials and community leaders. In the City of Norfolk, an aggressive plan to rehabilitate wetlands on city property has begun and consequently provided a unique learning opportunity for students from elementary age through graduate school. Restoration sites within Colley Bay in Norfolk, Virginia which is adjacent to Larchmont Elementary School and Old Dominion University have been identified. Elementary students have been growing the marsh grass, Spartina alterniflora, and will assist in the planting, while the ODU Biology Graduate Student Organization have made the technical plans and submitted all necessary permit applications. With funding from the City of Norfolk Wetlands Board and assistance from the Lafayette River Wetlands Partnership, this project promises to have significant positive impacts on the environmental quality and aesthetics of the area.

### **Environmental Science**

ESTABLISHMENT OF A CRITICAL THERMA MAXIMA (CTMAX) FOR THE MAYFLY ISONYCHIA BICOLOR (EPHEMEROPTERA). C.A. Sims, B.S. Echols, J. Brunkow, W. Nuckols and D.S. Cherry. Department of Biology, Virginia Tech. A study of Critical Thermal Maxima (CTMax) for the mayfly, Isonychia bicolor, began in April 2008 and extended to October 2009. Organisms were collected using d-frame dip nets and hand picking techniques from Sinking Creek, Giles County, Virginia. Mayflies were subject to gradual temperature change and monitored for behavioral physical consequences of the increased thermal stress were observed including sporadic swimming and ecological death, defined as the inability to cling to surfaces. Results were compared with past research in order to address accuracy. In general the results showed that the mayflies were more sensitive to thermal changes. Mayflies are often

used in laboratories as test organisms to assess environmental stressors, however specific conditions have not been established to keep this organism in the best condition for testing. It appears that 34°C is the CTMax for this species.

MODELING FISH SPECIES DIVERSITY IN FORESTED AND URBAN STREAMS: A BASELINE FOR CLIMATE CHANGE. Eugene G. Maurakis (1,2), David V. Grimes (3,1) Suzy Short (1), and Amanda Schutt (4,1). (1) Science Museum of Virginia, 2500 W. Broad St., Richmond, VA 23220 (2) University of Richmond, VA 23173, (3) VA Dept. Environmental Quality, Richmond, VA 23060, (4) Center for Environmental Studies, Virginia Commonwealth University, Richmond, VA 23284. Objectives are to model fish species richness, diversity and evenness in watersheds of Quantico Creek (forested watershed) and Cameron Run (urban watershed) using biological, physio-chemical factors, and land use and human population data per intradrainage stream order area. To date, 32 species of fishes (11 families) have been collected in 272 collections made from Nov. 2008-May, 2010. Overall, species richness, diversity, and evenness in forested areas are significantly higher than those in urban streams. Stream order, water depth, and month account for the variation in species richness in the forested watershed. In contrast, elevation and stream flow account for the variation in species richness in the highly modified stream beds of the urban watershed. Funded by the U.S. Department of Energy.

STORMWATER MANAGEMENT AND EDUCATION CENTER AT THE SCIENCE MUSEUM. Eugene G. Maurakis (1,2) and Todd Janeski (3), (1) Science Museum of Virginia, 2500 W. Broad St., Richmond, VA 23220 (2) University of Richmond, VA 23173, (3) VA Dept. Conservation and Recreation, 203 Governor, St., Richmond, VA 23219. We are developing a regional environmental site design demonstration, education, and training center. We are retrofitting a highly visible community facility with low impact development stormwater management practices, monitoring their effectiveness, and developing a training/certification program that showcases those measures. These include: bioretention facilities, a Bayscapes garden, tree box filters, porous pavement, rainwater harvesting system, and a green roof. We will evaluate their performance through a quality assured monitoring program. To achieve the scale necessary to demonstrate their applicability, we will conduct a sewershed low impact development retrofit survey of the entire sewer drainage area of Shockhoe Creek (8000 acres, about 2/3 of the City of Richmond CSO system) to the James River. Recognizing the effects of climate change on stormwater planning, we will conduct a 2nd survey utilizing predicted increases in frequency and intensity of storm and drought conditions. And applying a cost benefit analysis, we will demonstrate their benefits in relation to the cost to address damages incurred by uncontrolled stormflows. Funded by the National Fish & Wildlife Foundation.

THE IMPACT OF DEVELOPMENT ON SYMBIOSES FROM THE POTOMAC RIVER VALLEY TO THE CHESAPEAKE BAY. M. Aziz, A. Carpenter, D. Griffith, L. Kinne, and C. Milling, George Mason University. Regional development in the Potomac River Valley (PRV) has placed stressors on local symbioses affecting

ecosystem services. Symbiosis can be defined as "two or more forms of life that interact." Many symbiotic relationships that provide ecosystem services exist in the PRV. These symbioses include plants with pollinators, oysters with submerged aquatic vegetation, and mycorrhizae with plants. Their services include: water filtration and habitat restoration. The last twenty years has shown tremendous growth in population in the Northern Virginia region, with counties growing up to 96 percent. Development as a result of this population expansion has led to increased stressors including habitat degradation and fragmentation, sedimentation, toxic and organic pollutants, and changes to flow regimens. This phenomenon has the potential to fundamentally alter symbioses and the ecosystem services they provide to humans. It is unclear how long the PRV ecosystem can be maintained given the effects of these stressors, but it is unlikely that the resilience is absolute. Society has prospects to help the ecosystem: education and implementation of new policies and technologies could allow the natural symbiotic relationships to continue and recover, ultimately benefiting the PRV.

PRELIMINARY **ANALYSIS** OF BAY **FILTER** UNIT SUCCESS IN FREDERICKSBURG, VA. Michael L. Bass and Marion A. Cross, University of Mary Washington, Fredericksburg, VA. Virginia Department of Conservation and Recreation (DCR) evaluates and approves manufactured treatment devices (MTD's) deemed reasonable methods of prevention, control and/or treatment of storm water runoff. MTD's seeking certification for runoff quality control in Virginia will only be approved for total phosphorous (TP) removal at this time, requiring 50% TP removal for influent with TP concentrations ranging from 0.15 mg/L to 0.5 mg/L. Baysaver Technologies, Inc has applied for interim approvals to use the Bay Filter System to meet Virginia requirements for treating stormwater runoff. A monitoring program is intended to demonstrate through field testing that Bay Filter is capable of removing contaminants from stormwater runoff. Results will determine if the filter meets stormwater regulations. The field testing program will collect discrete samples from the influent and effluent of the BayFilter. These samples will be analyzed using standard EPA protocols for total suspended solids (TSS), particle size distribution (PSD), nutrients as well as metal concentrations. Removal efficiencies will be calculated based on this data using standard scientific methods. Precipitation and flow records will be taken during these events as well. The testing program is anticipated to take 12-18 months to complete and will include at least 15 qualifying storm events. BayFilter systems to be monitored will treat the stormwater runoff from Trinity Episcopal Church property in Fredericksburg, VA. Stormwater runoff from the paved area transports dissolved, colloidal, suspended and settleable solids in a heterogeneous mixture, which includes metals, organic compounds and nutrients.

REPORT OF MONITORING FOR SELECT WATER QUALITY PARAMETERS IN THE STORMWATER MANAGEMENT PONDS FOR A COMMERCIAL DEVELOPMENT. Michael L. Bass, Marion A. Cross and Leah N. Sumner. Earth and Environmental Sciences, Univ. of Mary Washington, Fredericksburg, VA. In the 1990's the Silver Company built Central Park in Fredericksburg, Virginia. In the process 6.9 acres of wetlands were destroyed and had to be reconstructed in compliance with

section 404 of the Clean Water Act. Storm water management ponds were built with the wetland benches around them within Central Park and a mitigation site was created along Massaponax Creek in Spotysylvania County. This study monitored the storm water management ponds of Central Park. This background data will be used as preliminary info on nutrient loads in these ponds when the new DCR regulations are in place. Water quality monitoring was conducted on the storm water management ponds and the migrated wetland site. Dissolved oxygen, pH, temp., and conductivity were taken on site. Water samples were taken and tested in the laboratory for nitrates, phosphates, alkalinity and hardness. Testing was done on the same day as the samples were taken. LaMotte test kits were used for each test, and the nitrate and phosphate levels were measured with a colorimeter. The storm water management ponds exhibited normal ranges in chemistry values, with high nutrient levels in the Best Buy, Kohl's and Upper Target ponds still not exceeding EPA maximum dose levels. These ponds also exhibit higher alkalinity and water hardness levels and are constructed with culverts of concrete that is weathering, which could contribute to the elevations. Preparations are being made to monitor rainwater and surface runoff prior to entering the stormwater management ponds.

THE EFFECT OF RAIN GARDENS ON RUN-OFF WATER BACTERIA LEVELS. J. G. Felthousen, E. Wallace & Dr. B. Kreutzer, Department of Biology, Marymount University, Arlington VA 22207. A rain garden is a landscaped depression, designed to improve water quality by absorbing and filtering harmful substances in run-off water. In previous studies, rain gardens removed petroleum and fertilizers from run-off water. This study examined the effect of rain gardens on coliform bacteria found in run-off water. Coliform bacteria are medically important microorganisms often found in contaminated run-off water. During or immediately after rainfall, water samples were collected from two sites, a local rain garden and an adjacent parking lot. Each water sample was immediately transported to the lab and plated on differential media. After incubation, the rain garden and parking lot plates were assessed for coliform and non-coliform colony forming units. According to the results, after the rain garden was flushed with initial spring rains, some coliform bacteria levels dropped. To draw further conclusions, sampling must continue at local rain garden sites throughout the year.

PRELIMINARY **ANALYSIS** OF BAY FILTER UNIT SUCCESS IN FREDERICKSBURG, VA. Michael L. Bass and Marion A. Cross, University of Mary Washington, Fredericksburg, VA. Virginia Department of Conservation and Recreation (DCR) evaluates and approves manufactured treatment devices (MTD's) deemed reasonable methods of prevention, control and/or treatment of storm water runoff. Virginia's stormwater management programs are implemented under: Virginia Stormwater Management Law and Virginia Stormwater Management Regulations. DCR maintains the authority to regulate BMP methods used in Virginia to control stormwater runoff under the Virginia Technology Assessment Protocol (VTAP). The assessment protocol deals with the MTA's that are designed for, reducing stormwater runoff volume, reducing peak runoff rate and/or and reducing total phosphorous (TP). The goal of the VTAP regarding runoff quality control is to determine how much a specific

MTD can remove total phosphorous (TP). MTD's seeking certification for runoff quality control in Virginia will only be approved for TP removal at this time, requiring 50% TP removal for influent with TP concentrations ranging from 0.15 mg/L to 0.5 mg/L. Additional requirements are 80% removal of TSS for influent with TSS concentrations ranging from 100 mg/L to 200 mg/L and > 80% removal of TSS for influent with concentrations greater than 200 mg/L. Baysaver Technologies, Inc has applied for interim approvals to use the Bay Filter System to meet Virginia requirements for treating stormwater runoff. Flow through the filter system in gravitydriven and self-regulating. The monitoring program is intended to demonstrate through field testing that Bay Filter is capable of removing contaminants from stormwater runoff. The field test will demonstrate the removal efficiencies attained by the system for TSS, TP, Cu, Zn and other pollutants. This will then be used to confirm that the system meets stormwater regulations which require the removal of a minimum 80% of the total suspended sediment load and treatment of nutrients to the maximum extent feasible. The field testing program will collect discrete samples from the influent and effluent of the BayFilter. These samples will be analyzed using standard EPA protocols for total suspended solids (TSS), particle size distribution (PSD), nutrients as well as metal concentrations. Removal efficiencies will be calculated based on this data using standard scientific methods. Precipitation and flow records will be taken during these events as well. The testing program is anticipated to take 12-18 months to complete and will include at least 15 qualifying storm events. BayFilter systems to be monitored will treat the stormwater runoff from Trinity Episcopal Church property in Fredericksburg, VA. Stormwater runoff from the paved area transports dissolved, colloidal, suspended and settleable solids in a heterogeneous mixture, which includes metals, organic compounds and nutrients. These constituents result from atmospheric deposition, traffic activities, vehicular wear, pavement degradation and deicing, landscape maintenance and littering. The nutrient load from the site is expected to vary seasonally.

REPORT OF MONITORING FOR SELECT WATER QUALITY PARAMETERS IN THE STORMWATER MANAGEMENT PONDS FOR A COMMERCIAL DEVELOPMENT. Michael L. Bass, Marion A. Cross and Leah N. Sumner. Earth and Environmental Sciences, University of Mary Washington, Fredericksburg, VA. In the 1990s the Silver Construction Company built Central Park in Fredericksburg, Virginia. In the process 6.9 acres of wetlands were destroyed and had to be reconstructed in compliance with section 404 of the Clean Water Act. In order to meet the requirements. storm water management ponds were built with the wetland benches around them within Central Park and a mitigation site was created along Massaponax Creek in Spotysylvania County. The purpose of this study was to monitor the storm water management ponds of Central Park. This background data will be used as preliminary info on nutrient loads in these ponds when the new DCR regulations are in place. Water quality monitoring was conducted on the storm water management ponds and the migrated wetland site. Dissolved oxygen, pH, temperature and conductivity were taken on site using a YSI model 85 field multimeter. Water samples were taken and brought back to the lab at the University of Mary Washington. The samples were tested for nitrates, phosphates, alkalinity and hardness. Testing was done on the same day as the

samples were taken. LaMotte test kits were used for each test, and the nitrate and phosphate levels were measured with a colorimeter. The storm water management ponds exhibited normal ranges in chemistry values, with high nutrient levels in the Best Buy, Kohl's and Upper Target ponds still not exceeding EPA maximum dose levels. These ponds also exhibit higher alkalinity and water hardness levels and are constructed with culverts of concrete that is weathering, which could contribute to the elevations. Preparations are being made to monitor rainwater and surface runoff prior to entering the stormwater management ponds.

### **Medical Science**

A BIOMARKER PANEL FOR NON-ALCOHOLIC STEATOHEPATITIS (NASH) AND NASH-RELATED FIBROSIS. Zobair M. Younossi<sup>1,2,3</sup>, Sandra J. Page<sup>2,3</sup>, Nila Rafiq<sup>1,2</sup>, Aybike Birerdinc<sup>2,3</sup>, Maria Stepanova<sup>1,2</sup>, Noreen Hossain<sup>2</sup>, Arian Afendy<sup>1,2</sup>, Zahra Younoszai<sup>1,2</sup>, Zachary Goodman<sup>4</sup> & Ancha Baranova<sup>1,2,3</sup>, <sup>1</sup>Center for Liver Diseases, Inova Fairfax Hospital, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>3</sup>Center for the Study of Genomics in Liver Diseases, Molecular and Microbiology Department, George Mason University, Fairfax, VA, <sup>4</sup>Armed Forces Institutes of Pathology, Washington, DC. Non-alcoholic Fatty Liver Disease (NAFLD) is one of the most prevalent forms of chronic liver disease worldwide. Patients with NASH and NASH-related fibrosis, both intermediate stages of NAFLD, are at increased risk for progressive liver disease. Liver biopsy is used to diagnose these stages but has inherent risks; thus, a non-invasive alternative is greatly needed. This study examines the performance of a new, serum-based biomarker panel for NASH and NASH-related fibrosis. Serum from patients with biopsy-proven NAFLD was assayed for markers associated with the pathology of NASH and fibrosis. Regression models predictive of NASH, NASH-related fibrosis and NASH-related advanced fibrosis were then designed and cross-validated. The resulting models had AUC values > 80%, indicating high sensitivity and specificity. Together, these models formed a biomarker panel for NASH and NASH-related fibrosis that had good performance and was easy to use. Further testing on larger populations is warranted.

EXPRESSION OF CYTOKINES AND GASTRIC PEPTIDES IN MORBIDLY OBESE PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE. Amanda C. Zirzow<sup>1,2</sup>, Michael Estep<sup>2</sup>, Noreen Hossain<sup>2</sup>, Zachary Goodman<sup>2</sup>, Hazem Elariny<sup>2</sup>, Vikas Chandhoke<sup>1</sup>, Ancha Baranova<sup>1,2</sup> & Zobair M. Younossi<sup>2</sup>, <sup>1</sup>George Mason University, Fairfax VA 22030 and <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health Systems, Falls Church. Non-alcoholic fatty liver disease (NAFLD) describes the spectrum of conditions ranging from simple steatosis, the accumulation of excessive intercellular fat in hepatocytes, to non-alcoholic steatohepatitis (NASH), which is marked by necroinflammation and hepatic fibrosis. Although simple steatosis is relatively benign, 10 to 15 percent of the population will progress to NASH. Currently, the only way to diagnose NASH or to assess the stage of fibrosis is by obtaining a liver biopsy, which is invasive, expensive, and associated with

potential risks. This lack of diagnostics is intolerable since NAFLD occurs in an estimated 25 to 30 percent of the US general population, and NASH is reported in 2 to 3 percent of the population. NAFLD is closely associated with obesity, a chronic inflammatory state. This study investigates the role of the gastric appetite regulating peptides Ghrelin and Obestatin as well as several inflammatory cytokines by measuring their expression in the context of NAFLD. Data generated by this work could have direct relevance to patient diagnosis and screening as well as advance scientific understanding pertaining to the complex regulation of appetite stimulation and suppression in the context of obesity related disease.

ALPHA-MELANIN STIMULATING HORMONE (α-MSH) AND MELANIN CONCENTRATING HORMONE (MCH) EXPESSION IN OBESITY AND OBESITY RELATED DISEASES. Massih Abawi<sup>1,2</sup>, Michael Estep<sup>2</sup>, Vikas Chandhoke<sup>1</sup>, Zobair M. Younossi<sup>2</sup>, & Ancha Baranova<sup>1,2</sup>. <sup>1</sup>George Mason University, Fairfax VA 22030 and <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health Systems, Falls Church. In humans, melanin is produced in melanocytes and a few other specialized cells of the body. Our lab has been the first to demonstrate that melanin biosynthesis pathway is functional in adipose tissue of morbidly obese subjects. Melanin biosynthesis is regulated by melanogenic peptides αMSH and MCH. The aim of this study was to assess circulatory levels of aMSH and MCH in morbidly obese patients with obesity related diseases. Clinical data and fasting serum samples were collected from 39 morbidly obese NAFLD patients at the time of the liver biopsy. All liver biopsies were interpreted by a single hepatopathologist and assessed for liver disease. Fasting serum was assayed for aMSH and MCH concentrations which were determined by sandwich ELISA. Cytokine concentrations were obtained by BioPlex Multi-plex assay. Circulating levels of MCH and aMSH displayed strong positive correlation (r=0.76, p<0.001). Concentrations of αMSH showed small but statistically significant positive correlations with IL-6 (r=0.36, p<0.05), and Kupfer cell inflammation (r=0.385, p<0.05). Circulating concentrations of MCH also showed positive correlations with IL-6 (r=0.32, p<0.05), and Kupfer cell inflammation (r=0.35, p<0.05). Circulating levels of MCH and aMSH significantly correlate with markers of inflammation and may participate in the pathogenesis of NAFLD.

A CALCINEURIN-DEPENDENT LOSS AND AN OVERGROWTH OF DENDRITIC SPINES AFTER TRAUMATIC BRAIN INJURY IN RAT. John N. Campbell, Brian Low, David R. Register, & Severn B. Churn, Dept. of Neurology, Virginia Commonwealth University, Richmond VA 23298. Traumatic brain injury (TBI) can cause cognitive dysfunction in the absence of cell death, likely due in part to changes in neuronal connectivity. Dendritic spines form most of the excitatory synapses in the brain, and thus are one measure of neuronal connectivity. A loss of dendritic spines has been reported after TBI in human tissue samples, but this effect and its underlying mechanisms have not before been examined in an experimental model. In the present study, a modified Golgi-Cox technique was used to investigate the effect of TBI on dendritic spines at 1 h, 24 h, and 1 wk after lateral TBI in the adult rat. Principal cell dendrites were sampled for spine density in layer II/III neocortex,

hippocampal CA1 and CA3, and dentate gyrus. By 24 h post-TBI, the density of pedunculated (thin or mushroom-shaped) spines had decreased by 30% in ipsilateral layer II/III neocortex (p<0.05; n=19), by 29% in ipsilateral CA1 (p<0.001; n=18), and by 23% in contralateral CA1 (p<0.01; n=12). Strikingly, this loss of spine density was prevented by a single, 1 h post-TBI administration of the calcineurin inhibitor, FK506. By 1 wk post-TBI in untreated subjects, dendritic spine density returned to control levels in some brain regions but increased above control levels in other regions (ipsi CA1; +52%, p<0.001, n=14; contra CA1; +43%, p<0.001, n=13; ipsi CA3; +34%, p<0.01, n=14; contra CA3; +25%, p>0.05, n=13). These data imply significant, bilateral changes in the synaptic circuitry of the laterally-injured brain. Research supported by Commonwealth Neurotrauma Initiative grant 07-302E to S.B.C.

HERMITAMIDE B: DISCOVERY OF A MARINE NATURAL PRODUCT SODIUM Eliseu O. De Oliveira<sup>1</sup>, Kristin M. Graf<sup>1</sup>, Kan Wang<sup>1</sup>, CHANNEL BLOCKER. Sivanesan Dakshanamurthy<sup>1</sup>, Milton L. Brown<sup>1</sup>, Manoj K. Patel<sup>2</sup>, & Mikell Paige<sup>1</sup>, <sup>1</sup>Drug Discovery Program, Georgetown University, Washington DC 20057 and <sup>2</sup>Dept. of Anesthesiology, University of Virginia, Charlottesville VA 22908. Marine natural products have historically been an important source of new drugs. The cyanobacterium Lyngbya majuscula present in tropical and sub-tropical waters produce a range of cytotoxic secondary metabolites. The lipopeptide hermitamide B was isolated from L. majuscula collected from deep-water coral reefs at Hermit Island Village, in Papua New Guinea. Because of its structural similarity with the jamaicamides, a family of sodium channel blockers isolated from cyanobacterium, we hypothesized that hermitamide B would also behave as a sodium channel blocker. We were delighted to find in our initial in vitro screen that hermitamide B displaces [3H]-BTX from sodium channels in a comparable manner with phenytoin (i.e. ~20%, at 10 μM), a clinically used sodium channel blocker. Subsequent electrophysiology experiments showed that hermitamide B significantly blocks the sodium current in HEK-293 cells that over express Na<sub>V</sub>1.2 sodium channels (>80% blockade at 1 μM). Herein, we present our total asymmetric synthesis of hermitamide B, using three major strategies: Keck allylation for chiral center formation, Johnson-Claisen condensation to set the E-olefin, and carbodiimide assisted coupling of lyngbic acid with the appropriate amine to give the final product. Yield was 8% over 7 steps with a >95:5 er.

THE IDENTIFICATION OF NOVEL RAT NITRIC OXIDE SYNTHASE 1 FIRST EXONS MAY LEAD TO A BETTER UNDERSTANDING OF DIABETES. Robert L. Murphy, Divya Bansal, Robert Lera, & Terrie K. Rife, Dept. of Biol. James Madison Univ., Harrisonburg VA 22807. The misregulation of nitric oxide synthase I (NOS1) has been linked to type-2 diabetes. Due to the difficulty of obtaining human tissue and controlling for environmental influences, many researchers use the rat model to study transcriptional changes in NOS1 during the pathogenesis of these diseases. However, the rat NOS1 gene has not been completely characterized, which prevents us from understanding which promoters might be directing disease-related changes in NOS1 expression. The better-characterized human NOS1 gene has twelve first exons with associated promoters (1a -11). The translation start site of the gene is found in the

second exon, which is common to each transcript resulting in the same functional protein. The rat NOS1 gene has known orthologs to the human first exons 1b, 1c, 1f, and 1g. However, these exons and associated promoters alone do not explain the changes in NOS1 expression that occur in rats with type-2 diabetes. Thus, rat orthologs to the other human first exons were hypothesized using genomics techniques. Two of the predicted first exons, orthologs to human 1h and 1k, were confirmed to initiate transcription in rats using reverse transcription, PCR, and Southern blotting and were then sequenced. The ortholog to human 1h was found to be expressed in the brain, intestine, kidney, retina and testis, but not in skeletal muscle, and the ortholog to first exon 1k was only expressed in brain. The next step of this research is to study how the transcription of these first exons is altered in diseased rats. This research was funded by a National Biological Honor Society research grant.

RESPONSIVE CHANGES TO NEONATAL GENISTEIN AND ESTRADIOL EXPOSURE IN THE POST-PUBERTAL MOUSE TESTIS AND EPIDIDYMIS: HISTOLOGICAL AND CELLULAR ANALYSIS. Nathan T. Derstine, Ben K. Ruth, & Roman J. Miller, Department of Biology, Eastern Mennonite University, Harrisonburg VA 22802. Effects of neonatal exposure to genistein or estradiol on the post-pubertal testis and epididymis were examined in Swiss mice, injected subcutaneously with control (no-hormone vehicle), estradiol (15µg / injection), or genistein (166µg / injection) every other day from post-natal day (PND) 2 through 14. Mice were necropsied on PND 39; testes and epididymides were prepared for histology. Compared with control, testis weights were reduced in genistein and estradiol groups by 19% and 39% respectively. Seminiferous tubules of genistein and estradiol mice had reduced percentage of tubular wall, total lumen space, and luminal spermatozoa compared to control. In the estradiol group the epididymis organ weightwas reduced by 43% from control and the mean tubular diameter was significantly increased by 7.3%. Spermatozoa counts were reduced by 74% in genistein-treated mice and were not found in the estradiol group. Numerous unusual cells were found in the epididymis tubular lumen of genistein mice. The unusual cells in the lumen are postulated to be immature sperm cells based on the herniated appearance of the seminiferous tubular wall and their close resemblance to primary and secondary spermatocytes. (Research supported in part by Daniel B. Suter Biology Program Endowment, Eastern Mennonite University.)

MURINE FERTILITY FOLLOWING NEONATAL EXPOSURE TO GENISTEIN AND ESTROGEN. Katrina J. Lehman, Jackson T. Maust, Brittany D. Kropf, Kristina R. Landis, and Roman J. Miller, Department of Biology, Eastern Mennonite University, Harrisonburg VA. To determine the effects of selected estrogens on the murine reproductive system, fertility assays were conducted following seven neonatal subcutaneous injections of control vehicle, genistein, or estradiol on postnatal days 2, 4, 6, 8, 10, 12, and 14. Experimental male mice (six control, four genistein, four estradiol) from the treatment groups were individually housed with non-injected control (NIC) female mice as young adults for ten days (postnatal days 58 through 68 ±2 days) to allow mating. Similarly, five control, five genistein, and five estradiol female mice

from the experimentally treated groups were individually housed with NIC male mice for the same time period to allow mating. After the ten days, male mice were removed from the cages. The numbers of mice per litter were recorded on the dates of parturition, about 21-25 days following the mating period. Litter weights and numbers were recorded again on days 15-19 following parturition. None of the injected males in the estradiol group produced any offspring. For the females, statistically significant reductions in fertility were observed in both the genistein-injected females and the estradiol-injected females, although one female mouse from each treatment group produced offspring. The genistein-injected male mice did not appear to have a significant reduction in fertility. Because phytoestrogens such as genistein are found in soy products, continued research is necessary to determine the effects of exposure on fertility and reproductive development. (Research supported in part by Daniel B. Suter Biology Program Endowment, Eastern Mennonite University.)

THE ROLE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN THE ACUTE EFFECTS OF ALCOHOL. Anton J. Dawson, M. Imad Damaj. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA. Tobacco and Alcohol are the two most commonly abused drugs in the world and there is high co-morbidity of addiction to these substances. Substantial evidence suggests that neuronal nicotinic acetylcholine receptors (nAChRs), the receptors to which nicotine binds, may contribute to certain aspects of alcohol dependence. Such evidence has been demonstrated in vitro and in vivo in animals and However, the underlying mechanisms of these in genetic studies in humans. interactions are still not well understood. Thus we initiated an investigation to uncover the specific nAChR subtypes involved in some of the many underlying mechanisms of ethanol-induced behaviors. We used a variety of approaches to investigate the modulating effects of nicotinic antagonists on such behaviors in C57BL/6J mice. The first approach was to study the acute effects of ethanol-induced loss of righting reflex (LORE), anxiolysis, and hypothermia. The results showed that the drugs Mecamylamine, Dihydro-β-Erythrodine, and Varenicline, in addition to knockout mice lacking the β2 subunit, significantly modulated sensitivity to the ethanol-induced LORE response. Varenicline also reduced ethanol-induced anxiolysis, while increasing sensitivity to the hypothermia response. In conclusion, we have added to data in the field suggesting the involvement of β2-containing nAChRs mediating some of the acute effects of alcohol. Future efforts will continue with additional antagonists and gene knockout mice to understand specific subunits including the largely unexplored a5 and α6 subunits and their relation to ethanol behaviors.

OPIOID AND GP-120 INTERACTIVE NEUROPATHOGENESIS IN HIV-1: ROLE OF CASPASE-3. <u>Kimberly, L. Samano</u> & Kurt F. Hauser. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. gp120, an HIV-1 coat protein, is an established neurotoxin and it is required for viral entry and infection, and its extracellular actions are toxic to microglia, astrocytes, and neurons. It is hypothesized that morphine will exacerbate gliosis and neuronal cell death caused by gp120 *in vivo* and this neuropathogenesis is proposed to occur through

a caspase-3 dependent mechanism. Studies will investigate the effect of morphine and HIV-1 on reactive gliosis of astrocytes and microglia via stereotaxic intrastriatal injection of gp120 into C57BL/6J and caspase 3 knockout (KO) mice. Gliosis will be assessed by co-localization studies performed via fluorescent microscopy probing for glial fibrillary acidic protein (GFAP) and  $\mu$  opioid receptor as well as Iba, with 3nitrotyrosine (3-NT for nitrosative stress). It is anticipated that μ opioid receptor expressing glia will be more vulnerable to insult versus cells lacking the receptor. Neuronal death will be verified by TUNEL assay to determine if morphine potentiates gp120-induced neuronal apoptosis and to explore if this effect can be alleviated through genetic deletion of caspase-3. Unexpectedly, preliminary results show that morphine administered to caspase-3 KO mice was lethal. This effect was abolished by pretreatment with naltrexone, strongly suggesting this interaction is opioid receptor mediated. Additional studies will be conducted to assess this novel finding and to investigate the mechanism(s) of morphine lethality. Collectively, these studies will add to the understanding of how morphine influences the neuropathogenesis of HIV-1 as well as explore the role of caspase-3 in this interactive comorbidity.

NAVIGATING THE MURKY WATERS OF NICOTINE AND ANXIETY: CONTRIBUTIONS OF THE B2 SUBUNIT. Shawn M. Anderson & Darlene H. Brunzell. Virginia Commonwealth University School of Medicine, Richmond VA 23298. Nicotine, the primary psychoactive agent in tobacco, exerts its effects by binding to nicotinic acetylcholine receptors (nAChR) in the brain, with \( \beta 2 \) containing nAChRs (\( \beta^2 \) nAChRs) having the highest binding affinity for nicotine. \( \beta^2 \) nAChRs are significantly upregulated in the brains of smokers and rodents following extended exposure to nicotine. Although smokers report that they smoke to relieve anxiety, controlled studies suggest that repeated exposure to nicotine increases anxiety behavior. The purpose of these studies was to assess the role of \$2\*nAChRs in anxiety-like behavior. Male \$2\*nAChR knockout (?2KO) mice on a C57BL6 background and their wildtype (WT) counterparts were tested in a light-dark box assay after i.p. injection of 0, 0.01, 0.05, 0.1, or 0.5 mg/kg nicotine in 0.9% saline. ANOVA tests revealed a significant interaction of treatment and genotype for behavioral measures of latency and light-chamber exploration. WT animals that received 0.5 mg/kg nicotine showed significant increases in latency to leave dark chamber (p < 0.05) and decreases in locomotor activity in the light chamber (p < 0.05) compared to controls. These effects were not seen in ?2KO animals. These data suggest that \( \mathrm{B}2\*nAChRs \) contribute to movement/exploratory behavior in an aversive environment. Consequently, it appears that B2\*nAChRs contribute to the anxiogenic effects of nicotine administration in the light-dark assay and suggest a potential mechanism for elevated anxiety behavior in smokers. This work was supported by a Jeffress Memorial Trust research grant J-951 and a NIDA small grant project award DA005274.

THE FULL AGONIST WIN55, 212-2 EXERTS GROWTH INHIBITORY EFFECTS THROUGH A CANNABINOID RECEPTOR INDEPENDENT MECHANISM. Sean M. Emery, David A. Gewirtz & Aron H. Lichtman, Virginia Commonwealth University, Richmond VA. Cannabinoids are known to inhibit the growth of a variety

of cancer cells in vitro, including those derived from glioma, breast, prostate, and lung tumors, among others. In the present study, we tested whether WIN55, 212-2 (WIN2), a synthetic cannabinoid that acts as a full agonist at both known cannabinoid receptors, CB<sub>1</sub> and CB<sub>2</sub>, produces antiproliferative effects in both human (MCF-7 and MDA-MB-231) and murine (4T1) breast tumor cells. WIN2, but not its inactive stereoisomer WIN55, 212-3 (WIN3), elicited inhibitory effects on cancer growth, suggesting that these antiproliferative effects are due to the drug acting at a specific site of action. Interestingly, the highly selective CB<sub>1</sub> and CB<sub>2</sub> antagonists, Rimonabant and SR144528, respectively, did not block the effects of WIN2, either alone or in combination. Instead, each cannabinoid receptor antagonist enhanced the growthinhibitory actions of WIN2 in each cell line. Both CB, and CB, are G protein coupled receptors (GPCRs), predominantly activating Gi/Go subtypes. Accordingly, we tested whether pertussis toxin, which inhibits Gi/Go proteins, would prevent WIN2's antiproliferative actions, but the treatment was unable to prevent WIN2's actions in any of the breast cancer cell lines. Taken together, these data suggest that WIN2 inhibits breast cancer cell growth through a non-GPCR mechanism of action, but the stereoselective-dependent nature of this effect suggests specific site(s) of action. Experiments are currently underway to determine the underlying mechanism of action for the antiproliferative effects of WIN2.

MODULATION OF GLIAL FUNCTION BY MORPHINE AND THE HIV-1 PROTEIN GP120. Elizabeth M. Podhaizer & Kurt F. Hauser, Dept. of Pharmacology and Toxicology, Virginia Commonwealth Univ., Richmond VA 23298. Opioid abuse, through injection drug use is tightly linked to HIV-1 infection through both the spread of the disease and by exacerbation of disease progression culminating in HIV-1 encephalitis (HIVE) and neurocognitive impairments. CNS glia are intimately involved in the dual effects of opioids and HIV-1, as glia, unlike neurons, are infected by HIV-1 and additionally release inflammatory and modulatory substances that can activate neighboring glia as well as interfere with neuronal function. Previous work has shown that astrocytes are directly involved in morphine's toxicity. Thus, we hypothesized that opioids acting directly through astrocytes, dysregulate glial function and lead to interactive neurotoxicity in the presence of HIV-1 infection. To address this hypothesis, we examined important convergent signaling events of two Gi/o coupled receptors, MOPr and CCR5, the receptors for morphine and the envelope glycoprotein, gp120 respectively. Opioids and HIV-1 proteins elevate intracellular ROS in astrocytes as well as promote increases in intracellular calcium levels independently, which are linked to inflammatory signaling. Morphine, but not gp120 altered p-ERK levels with morphine decreasing p-ERK over a 60 minute period, suggesting that opioids impair the proliferative function of astrocytes. Additionally, agonist selectivity is present between gp120 and the endogenous ligand to CCR5, RANTES which elevated p-ERK over 60 minutes. These results suggest that signaling through opioid and chemokine receptors by morphine and gp120 have similar points of convergence where interactive signaling will be examined.

ARTEMIS ENDONUCLEASE: A CRITICAL REINFORCEMENT TO THE G1 DNA REPAIR ARMAMENT. Susovan Mohapatra<sup>1</sup>, L.F. Povirk<sup>1</sup>, Imran Khan<sup>2</sup>, M.K.Stillion<sup>2</sup> & S.M. Yannone<sup>2</sup>, <sup>1</sup>Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond Va and <sup>2</sup>Dept. of Genome Stability, Lawrence Berkeley Laboratory, Berkeley, Ca. DNA double strand breaks (DSB) are the most significant lesions resulting from radio/chemotherapeutic intervention of cancer. Nonhomologous end-joining (NHEJ) is considered to be a critical DNA repair pathway and mutations in the NHEJ factor Artemis have been implicated in radiosensitive severe combined immunodeficiency (RS-SCID) or Athabascan SCID in humans, as well as increased risk of lymphoma in some settings. Prior in vitro studies showed that Artemis has a DNA-PK-dependent endonuclease activity at DNA ends. To assess the possible role of this endonuclease activity in chemo/radioresistance, patient-derived Artemis-deficient CJ179 fibroblasts were stably complemented with lentiviral vectors expressing either wild-type or D165N Artemis, a mutation that eliminates its endonuclease activity. As determined by clonogenic survival assays, expression of wild-type Artemis but not D165N mutant conferred approximately two-fold resistance to ionizing radiation, as well to the radiomimetic agents bleomycin and neocarzinostatin in CJ179 cells. Measurements by γ-H2AX, 53BP1 focus formation and pulse-field gel electrophoresis (PFGE) assays suggested a repair defect (10-20%) in Artemis-deficient and D165N Artemis mutant cell lines, but not in wild-type Artemis-complemented cells, particularly at 6-18 hr post-irradiation. These results, combined with previous in vitro studies, suggest that resolution of terminally blocked DNA ends by the endonuclease activity of Artemis may be its biologically relevant function.

GENOMIC ANALYSIS OF THE ETHANOL DEPRIVATION EFFECT. Jonathan A Warner & Michael F. Miles, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA. The potential for relapse into abusive modes of substance use is of paramount concern in recovery from any type of substance addiction. Alcoholism presents significant challenges to the addict due to its strong heritability and the pervasive legal availability of alcohol in most industrialized societies. The ethanol deprivation effect (EDE), also known as the alcohol deprivation effect, models relapse behavior of human alcoholics, and manifests in mice as an increase in ethanol consumption and preference following forced abstinence, which is attenuated by naltrexone and acamprosate. Several transcripts previously identified by microarray analysis of nucleus accumbens as significantly regulated by ethanol deprivation were confirmed with quantitative PCR. These transcripts code for proteins involved in a diverse range of cellular functions, including calcium regulation, mitochondrial localization, RNA interference, and chromatin modification. Because repeated deprivations have been shown to increase the magnitude of the EDE, a longterm "binge" model with repeated deprivations was used to obtain further samples for genomic analysis. After one month of habituation and one month of uninterrupted ethanol access the mice were subjected to eight cycles of six days of ethanol deprivation followed by one day of reinstatement. This model produced a significant and sustained increase in ethanol consumption and preference for ethanol over water following the

first deprivation period, and it should prove useful in further exploration of the molecular mechanisms of both development and maintenance of the EDE, as well as in testing long-term efficacy of therapeutics for alcoholism.

PAIN-RELATED DEPRESSION OF INTRACRANIAL SELF-STIMULATION IN RATS: EFFECTS OF THE DELTA OPIOID AGONIST SNC80 AND THE PSYCHOMOTOR STIMULANT COCAINE. Marisa B. Rosenberg<sup>1</sup>, John E. Folk<sup>2</sup>, Kenner C. Rice<sup>2</sup> & S. Stevens Negus<sup>1</sup>, <sup>1</sup>Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA; <sup>2</sup>Chemical Biology Research Branch, NIDA/NIAAA, Rockville, MD. Pain is associated with a stimulation of some behaviors (e.g. withdrawal responses) and a depression of other behaviors (e.g. feeding, locomotion, responding maintained by many types of positive reinforcement). We have argued that analgesic drug development may benefit from complementary evaluation of drug effects on both pain-stimulated and pain-depressed behaviors. In this study, intraperitoneal injection of dilute lactic acid (1.8% in a volume of 1ml/kg) was used as a noxious stimulus in rats to stimulate a stretching response and to depress intracranial self-stimulation (ICSS) of the median forebrain bundle. The delta opioid agonist SNC80 (1-10 mg/kg, i.p.) dose-dependently blocked both acid-stimulated stretching and acid-induced depression of ICSS without altering control ICSS in the absence of the noxious stimulus. In contrast, cocaine (1-10 mg/kg i.p.) blocked acid-induced depression of ICSS only at doses that also facilitated control ICSS, and cocaine had no effect on acid-stimulated stretching. Flupenthixol (0.01-1 mg/kg, i.p.) blocked acidstimulated stretching but also decreased control ICSS and only exacerbated acidinduced depression of ICSS. Thus, the antinociceptive effects of SNC80 could be dissociated from the non-selective stimulant effects of cocaine and the non-selective depressant effects of flupenthixol. Supported by NIH grants RO1-DA11460 and R01-NS070715.

ROLE OF INSULIN RESISTENCE IN PCOS AND IMPLICATIONS FOR DEVELOPMENT OF METABOLIC SYNDROME AND HEPATOSTEATOSIS. Aybike Birerdinc<sup>1,2</sup>, Nandita Niranjan<sup>1</sup>, Noreen Hossain<sup>2,3</sup>, Arian Afendi<sup>2,3</sup>, Vikas Chandhoke<sup>1,2</sup>, Ancha Baranova<sup>1,2</sup> & Zobair Younossi<sup>1,2,3</sup>, <sup>1</sup>Molecular and Microbiology Dept. and Center for the Study of Genomics in Liver Diseases, George Mason Univ., Fairfax, VA 22030, <sup>2</sup>Translational Research Institute, Inova Health System, Falls Church, VA 22042 and <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA 22042. Polycystic ovarian syndrome is a common disorder observed in women aged mainly in the reproductive age bracket: from 12-45 years. PCOS is also associated with a number of other pathological features such as obesity, insulin resistance, and disregulation in lipid metabolism. In this study we attempt to determine the genetic commonalities between PCOS and NAFLD as both disorders have the hallmarks of Metabolic Syndrome. For this study 12 patients with diagnosed PCOS and 12 patients with confirmed lack of PCOS were selected. The two cohorts were carefully matched for clinical parameters such as presence of liver disease, BMI and age. These cohorts will be profiled by qPCR arrays to determine gene expression, by ELISA assays

to determine protein abundance, and microRNA panels to determine the involvement of microRNA's.

#### POSTERS

THE INDUCTION OF HEAT SHOCK PROTEIN 72 AT SPECIFIC HYPOTHERMIC INTERVALS IN AN ISOLATED AND PERFUSED RAT HEART MODEL-IMPLICATIONS FOR CARDIAC TRANSPLANTATION. E. Taylor<sup>1</sup>, I. Danelisen<sup>1</sup>. V. Sivakumaran<sup>2</sup>, J.E. Mahaney<sup>1,2</sup>, R.P. Wyeth<sup>1</sup>, <sup>1</sup>Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060 <sup>2</sup>Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Maintaining cardiac viability is a significant determinate in, and limitation to, cardiac transplantation. Current technology restricts the use of donor hearts due to increasing injury suffered to explanted hearts as time to implantation increases. Thus, the time span from procurement to implantation is greatly limited (~4 hours). Heat shock proteins (HSPs) contribute to cellular survival. Understanding HSP's role in cooling and rewarming of explanted hearts may increase the allowable time from explantation to implantation by protecting vital cardiac proteins from denaturation during transplantation. To test this hypothesis, male and females rat hearts were cannulated, stabilized, and perfused for 30 min at target temperatures. Caspase and HSP 72 were quantitated from myocardial homogenates. Myocardial injury, as caspase expression, was greater at the extremes of thermal stress as was myocardial protection, as HSP 72 expression. Furthermore, females expressed significantly more HSP 72 than males at hypothermic conditions. Our preliminary data suggest that HSP 72 is induced by thermal stress and that females are more capable of preventing protein denaturation at hypothermic conditions than are males.

A RETROSPECTIVE STUDY OF THE DISPARITY OF HEALTHCARE IN SOUTHWEST VIRGINIA BASED ON THE PRESENTATION OF ACUTE MYOCARDIAL INFARCTION AND ASSOCIATED MORTALITY IN PATIENTS LESS OR EQUAL TO 50 YEARS OF AGE. E. Taylor<sup>1</sup>, J. Powers<sup>1</sup>, R.P. Wyeth<sup>1</sup>, National Center for the Analysis of Healthcare Data<sup>1</sup>, Laboratory for Interdisciplinary Statistical Analysis<sup>2</sup>, <sup>1</sup>Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060, <sup>2</sup>Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Patients 50 years or less of age from southwestern (SW) were compared to patients residing in the remaining health districts of Virginia presenting with initial myocardial infarction (MI) with and without mortality. A total of 2280 patients were identified. The prevalence of initial nonfatal MI was 48.9/100K in SW Virginia and 37.7/100K in the rest of the Commonwealth. The prevalence of fatal MI was 7.0/100K in SW Virginia and 2.6/100K in the rest of the state. Neither prevalence of fatal nor nonfatal initial MI was statistically significant when SW Virginia patients were compared to patients from the remaining Commonwealth. There were however significant differences within mean household income and the population density. The mean income was \$55,846 in SW Virginia as compared to \$81,235 in the remaining Commonwealth, while the population density was 95 persons/1000 sq mile within SW Virginia and 258 persons/sq mile within the remaining Commonwealth.

# **Natural History & Biodiversity**

REMOVAL OF MAMMALIAN PREDATORS REDUCES PREDATION RATE ON ARTIFICIAL BEACH-NESTS ON THE VIRGINIA BARRIER ISLANDS. Raymond D. Dueser<sup>1,2</sup>, Joel D. Martin<sup>2</sup>, Nancy D. Moncrief<sup>1</sup> & John H. Porter<sup>3</sup>, <sup>1</sup>Virginia Museum of Nat. Hist., 21 Starling Ave., Martinsville, VA 24112, Dept. of Wildland Res., Utah State Univ., Logan, UT 84322, <sup>3</sup>Dept. of Env. Sci., Univ. Virginia, Charlottesville, VA 22903. We compared predation rates on eggs in artificial scrapes on (1) Metompkin and Parramore islands in August 2003 and on (2) Parramore Island before (2003) and after (2004) an intensive campaign to remove mammalian predators. On each island, we established 100 scrapes at randomly-selected points along a 4-km transect oriented parallel to the beach, above the high tide line. Each scrape was stocked with a "clutch" of 1 Japanese quail egg and 1 clay (Plasticene) egg. Each nest was monitored and restocked daily for 4 days. Metompkin was thought to be free of raccoons and red foxes in 2003, while Parramore harbored large numbers of both species. Mammalian predation rates were higher on Parramore (~99% per day) than on Metompkin (~0%). Nevertheless, gulls and ghost crabs depredated ~19% of the nests per day on Metompkin. We repeated this trial in 2004, using the same nest stations. Metompkin harbored 1-2 raccoons at this time, while Parramore still harbored both species even after a large number of raccoons and red foxes had been removed in autumn 2003. Mammalian predation rates on Parramore (18% per day) were still higher than on Metompkin (~0% per day), but were reduced dramatically from 2003. Once again, gulls and ghost crabs depredated ~6% of the nest per day on Metompkin. These results suggest that mammalian predation management has significant potential for reducing nest predation on islands.

THE BIRDS IN BETWEEN: PRODUCTIVITY OF EARLY SUCCESSIONAL BIRDS IN THE VIRGINIA PIEDMONT. Erica M. Rutherford<sup>1</sup>, Mark L. Fink<sup>1</sup>, Alix D. Fink<sup>1</sup>, & Michael D. Collins<sup>2</sup>, <sup>1</sup>Dept. of Biological and Environmental Sciences, Longwood University, <sup>2</sup>Dept. of Biology, Hampden-Sydney College. Some earlysuccessional species listed as being of global conservation concern, including the Prairie Warbler (Dendroica discolor), occur at high abundances in the Virginia Piedmont. Effective stewardship of those species requires understanding of how they are affected by management activities in the landscapes. We examined factors affecting reproductive success in a suite of six early successional species inhabiting in a variety of managed regenerating forests. In three seasons (2004, 2005, 2008), we located a total of 178 nests of the species suite: Prairie Warbler, Yellow-breasted Chat (Icteria virens), Field Sparrow (Spizella pusilla), Eastern Towhee (Pipilo erythrophthalmus), Indigo Bunting (Passerina cyanea), and Brown Thrasher (Toxostoma rufum) (and others; 10 species in total). Additionally, we measured habitat variables at two spatial scales: nest site and habitat patch. Simple nest success was 47% overall, with rates for focal species ranging from 53% for Yellow-breasted Chat (24 of 45) to 13% for Eastern Towhee (2 of 15 nests). Parasitism by Brown-headed Cowbirds (Molothrus ater) was relatively uncommon, occurring in 8% of total nests. Further analyses will use an

Information Theoretic Approach to evaluate factors influencing nest success, and survival models will be used to determine rates of daily survival. However, these preliminary estimates of simple nest success provide insights into the productivity of birds in these regenerating sites, as abundance is often the only indicator cited in their valuation.

DIVERSITY OF NON-APIS BEES IN SOUTHWEST VIRGINIA CROP LANDS. Nancy L. Adamson, Donald E. Mullins & Richard D. Fell, Entomology Dept., Virginia Tech, Blacksburg, VA 24061. Native bees provide the majority of crop pollination for some crops in the mid-Atlantic region. Little is known about native bee crop pollinators in Virginia, other than squash, bumble, and mason bees. This presentation highlights the abundance and diversity of non-Apis bees pollinating apples, blueberries, caneberries, and cucurbits in southwest Virginia during the 2008 and 2009 growing seasons, including six state records--Bombus sandersoni Franklin, 1913; Coelioxys rufitarsis Smith, 1854; Holcopasites calliopsidis (Linsley, 1943); Lasioglossum apocyni (Mitchell 1960); Melissodes communis Cresson, 1878; and Triepeolus simplex Robertson, 1903. As part of an on-going monitoring effort managed by the U.S. Geological Survey, the research provides baseline data to understand long-term population trends and to enhance land and farm management based on relationships between bee species richness and vegetation surrounding farm land. (Supported by a pollination research grant to the Virginia Cooperative Extension from the Virginia General Assembly and by the Virginia Tech Graduate Student Assembly's Research and Development Program and Travel Fund.)

SURVEY OF LOTIC DRAGONFLY SPECIES OF HANOVER COUNTY, VA. Allyson Lackey<sup>1</sup> & Leigh Adams<sup>2</sup>, <sup>1</sup>Biology Department, Virginia Commonwealth University, Richmond, VA 23298, <sup>2</sup> Biology Department, J. Sargeant Reynolds Community College, Richmond, VA 23285. Authors report as many as 27 lotic species might be found in this county. The collection and identification of adult dragonfly species (Odonata) found in the lotic areas of Hanover County, Virginia was conducted to verify the dragonflies found in this location. Preliminary field collections were made from two different rivers (Pamunkey and the South Anna) systems within Hanover County; one on each side of the county, occurring during the months of June through July in 2009. Adults of Anax junius, Gomphus lividus, Gomphus vastus, Arigomphus villosipes, Stylurus amnicola, Dromogomphus spinosus, Progomphus obscures, Libellula Lydia, Libellula vibrans, Libellula pulchella, Libellula incesta, and Libellula cyanea were collected and verified on the surveyed streams of Hanover County, Virginia, the summer of 2009.

SMALL MAMMALS FROM A CLOUD FOREST IN THE MONTAÑAS DE CUILCO, HUEHUETENANGO, GUATEMALA. John O. Matson<sup>1</sup>, Walter Bulmer<sup>2</sup>, Ralph P. Eckerlin<sup>2</sup>, Hayley Lanier<sup>3</sup> & Neal Woodman<sup>4</sup>, <sup>1</sup>Dept. of Biol. Sci., San Jose State Univ., San Jose, CA 95192, <sup>2</sup>Division of Nat. Sci., Northern Virginia Community College, Annandale, VA 22003, <sup>3</sup>University of Alaska Museum, Univ. of Alaska, 907

Yukon Dr., Fairbanks, AK 99775, <sup>4</sup>USGS, Patuxent Wildlife Research Center, National Museum of Nat. Hist., Smithsonian Institution, Washington, DC 20013. We surveyed the remnant mixed hardwood/coniferous cloud forest at elevations ranging from 2950m to 3160m at El Retiro, in the isolated Montañas de Cuilco, Huehuetenango, western Guatemala. Removal trapping for 4 days each in July 2008 (wet season) and January 2009 (dry season) resulted in 106 captures representing 6 species of shrews and rodents. This diversity of small mammals is the lowest that we have recorded from a Guatemalan cloud forest, compared to 10-15species at other localities. Based on capture rates, the species in order of relative abundance in the small mammal community are *Peromyscus beatae sacarensis* (n=45), *P. guatemalensis* (n=34), *Reithrodontomys microdon* (n=9), *R. sumichrasti* (n=7), *Sorex saussurei* (n=6) and *R. mexicanus* (n=5). The low species diversity may result from habitat destruction by recent large-scale fires and by logging for firewood and lumber. Habitat loss may have direct effects, but also leads to fragmentation that may restrict reinvasion after fire. This represents the first collection of small mammals from this mountain range.

# **Psychology**

GENDER, ETHNICITY, & ORGAN DONATION. Daniel Baughn<sup>1</sup>, Stephen M. Auerbach<sup>1</sup>, & Laura A. Siminoff<sup>2</sup>. <sup>1</sup>Department of Psychology, Virginia Commonwealth University, Richmond, VA 23284 and <sup>2</sup>Department of Social & Behavioral Health, Virginia Commonwealth University, Richmond, VA 23298. Understanding the factors that influence the procurement coordinator (PC) and the family at the time of organ donation may be one way to increase the rate of donation. Using an analogue format, this study examined the interpersonal behavior of PCs and simulated families during the donation request process. Interpersonal processes were assessed using behavioral ratings by independent observers using the Impact Message Inventory (IMI), the Participatory Style of Physician Scale (PSPS), and the Siminoff Communication Content and Affect Program (SCCAP). Three-way ANOVAs were conducted to evaluate the effects of gender of PC, ethnicity of PC, and ethnicity of family on the interactional variables. There was a significant PC gender × scenario (scn) interaction effect on IMI Affiliation, F(1,25)= 6.65, p<.02. There was a significant PC gender × ethnicity interaction effect on IMI Control, F(1, 25)=4.68, p<.04. There was also a significant PC gender × ethnicity interaction effect on the Shared Decision Making subscale of the PSPS, F(1,25)=5.83, p<.02. There was a significant PC ethnicity × scn interaction effect on the Positive Affect scale of the SCCAP, F(1, 25)=5.52, p<.03. Implications for the field of organ donation and the training of procurement coordinators are discussed.

IMPACT OF EMOTIONAL AROUSAL AND SECONDARY TASK MODALITY ON PERFORMANCE. Rachel R. Phillips & Poornima Madhavan, Dept. of Psych, Old Dominion University, Norfolk VA 23529. In order to examine the effect of different modality distractors (visual or auditory) of differing affect (positive or neutral) on performance participants completed a luggage screening task with and without a secondary task in one of four conditions (positive-visual, positive-auditory, neutral-

visual, neutral-auditory). In the auditory condition, positive affect was induced with Irish drinking songs and neutral was attained using Native American Drum music; for the positive-visual condition the lyrics were transcribed and in the neutral-visual condition symbols were substituted. 2 (distraction; present vs. absent) x 2 (affect: positive vs. neutral) mixed ANOVAs for hit rate (H), false alarm rate (FA), and confidence (C) in the auditory and visual conditions revealed that participants in the auditory condition had fewer false alarms when distracted versus undistracted. This indicated that participant performance improved as participants were less likely to incorrectly identify a target. In contrast, participants in the visual condition demonstrated a decrease in hits indicating that they were less likely to correctly detect a target. Between-subjects ANOVAs for H, FA, and C when distracted in the auditory condition revealed no significant differences between the positive and neutral conditions. Similarly, 2 (modality: visual vs. auditory) x 2 (affect: positive vs. neutral) between-subjects ANOVAs for H, FA, and C indicated no significant differences between any of the conditions. Results suggest that music is beneficial to visual search performance when compared to no stimulation. However, performance while distracted did not vary as a result of affect or modality.

INTENTIONAL FORGETTING OF AUTOBIOGRAPHICAL MEMORIES IN UNIVERSITY STUDENTS: THE IMPACT OF PERSONAL RELEVANCE ON THE DIRECTED FORGETTING EFFECT. <u>Tiffany M. Steinhagen</u> & Elaine M. Justice, Dept. of Psychology, Old Dominion University. This study examined the effect of personally relevant information on the directed forgetting of autobiographical memories. College and neutral-themed words were presented to an undergraduate population in conjunction with the Motivation Scale Learning Questionnaire. The degree to which a student's internal motivation for academic success influenced the directed forgetting effect was examined. Participants showed greater recall of college-themed words than neutral-themed words. Results indicated that individuals with high motivation were less likely to forget college-themed words under the forget instruction.

EXAMINING DISCREPANCIES BETWEEN STATED AND REVEALED PREFERENCES DURING INTERNET SEARCH. Molly M. Liechty & Poornima Madhavan, Old Dominion University, Norfolk VA 23529. Using an eye tracker we examined decision-making processes during an internet search task. Specifically, we studied the intrinsic factor of gender and extrinsic aspects of a website (or, negative externalities). Twenty-five undergraduate students browsed a simulated real estate website where they viewed photographs of ten houses, each with six rooms. We manually altered four homes to reveal Level 1 externalities (or, properties that can easily be changed) such as pink paint on the wall, and Level 2 externalities (or properties that cannot easily be changed) such as power lines in front of the house. The relationship between "stated preferences", or preferences that are verbalized, and "revealed preferences," or preferences that are revealed from an actual decision was analyzed as a function of negative externalities and participants' gender. Average dwell times, fixation durations/counts, and saccade counts/amplitudes were compared to participants' stated preferences on general and home specific surveys. Results revealed

that men demonstrated a more aggressive search pattern than women, with a greater number of saccades with shorter saccade amplitudes for the former. Data also suggested that a discrepancy exists between stated and revealed preferences. Although participants initially stated that they disliked a room, their eye movement indices did not reflect this trend indicating that subjective verbalizations were discrepant from actual internet search patterns.

FOR WHOM THE BELL TOLLS: **ACADEMIC** BEHAVIORS, REGULATION, AND PACED DRINKING IN ACOA AND NON-ACOAS. Gabrielle M. D'Lima & Michelle L. Kelley, Department of Psychology, Old Dominion University, Norfolk, VA 23529. The present study examined whether meeting criteria for being the adult child of an alcoholic (ACOA) explained differences in alcohol consumption and consequences experienced by college students (N = 127) during their first semester. Further, this research sought to identify possible mediators (i.e., pasthedonistic time orientation, future time orientation, self-pacing, alcohol interference with academic behaviors and general self-regulation) that weaken the direct relationship between ACOA status and alcohol consumption and alcohol consequences. Multiple hierarchical regressions were conducted to determine if ACOA status would explain additional unique variance in alcohol consumption and consequences beyond the mediating factors. ACOA status remained a significant predictor of alcohol consumption and consequences even after accounting for variance explained by the pertinent mediators. These results extend previous research by indicating the importance of variables that may mediate the relationship between ACOA status and alcohol use and alcohol-related consequences. Additionally, these results suggest that rather than simply comparing drinking outcomes in ACOAs as compared to non-ACOAs, it may be necessary to identify specific behaviors that may result in increased drinking behavior.

HERPES STIGMA ATTITUDES IN COLLEGE UNDERGRADUATE STUDENTS. Anna K. Harrington & Valerian J. Derlega, Dept. of Psychology, Old Dominion University, Norfolk, VA. Approximately one in five people in the United States has genital herpes. Previous research conducted with college student participants showed that those with more general knowledge concerning the disease were less likely to hold negative attitudes toward persons infected with genital herpes. Using the 10 item Rosenberg Self-Esteem scale (r = .85), the author found no interaction between self-esteem and negative attitudes concerning herpes. While the correlation between knowledge of genital herpes and more accepting attitudes towards persons with genital herpes was supported (p = .037), there was no correlation between negative attitudes and self esteem (p = ns).

THE INFLUENCE OF HIGHLY EMOTIONAL FACES ON THE ATTENTIONAL BLINK. <u>Brittany N. Pizzano</u> & Hilary E. Stebbins, Dept. of Psych., Virginia Wesleyan College, Norfolk VA 23502. The anger superiority effect demonstrates that angry faces capture attention and are identified more quickly relative to happy and neutral faces. Little is known, however, regarding the extent and duration of the attentional capture

of angry faces. The present study utilized an attentional blink paradigm in which participants were asked to identify two emotional target stimuli (e.g. female faces) out of a series of neutral distracter stimuli (e.g. male faces). The emotional expression of the first target was manipulated (happy, angry, or neutral) and it was predicted that greater attentional capture by the angry face would result in lower ability to process and identify the second target, resulting in an attentional blink. A total of 36 undergraduate students (28 female, 8 male) participated in this study. Analyses revealed an effect of both gender and emotion of the target stimuli. Happy faces produced the largest attentional blink for both male and female target trials. Angry faces were also found to produce an attentional blink for male but not female targets. These results suggest that greater attentional capture may result from general emotional arousal rather than a threat or negativity bias and that target gender is an important factor in processing facial expressions of emotion. This study was funded by the Virginia Academy of Science.

PSYCHOLOGY FOR NATIONAL SECURITY: IMPLEMENTING PSYCHOLOGICAL PRINCIPLES TO IMPROVE TRAINING OF AIRPORT SECURITY SCREENERS. Poornima Madhavan, Department of Psychology, Old Dominion University, Norfolk, VA 23529. This research examined the hypothesis that stimulus diversity during training can positively influence transfer of learning in complex tasks such a luggage-screening. Participants (n = 48) detected the presence of threat objects in x-ray images of airline passenger luggage. Training stimuli varied as a function of (1) categorical diversity (few vs. several categories of threat objects), and, (2) exemplar diversity (few vs. several exemplars within each category). Participants transferred this learning to a scenario where they encountered novel targets. High categorical diversity led to highest hit rates, fewest false alarms and fastest detection during transfer, which was not significantly influenced by exemplar diversity. However, high exemplar diversity negatively impacted transfer when categorical diversity was low, leading to the fewest hits and slowest response times. The results have implications for designing training modules for luggage-screening personnel in a manner that capitalizes on natural human cognition.

EXPLORING THE ASCH PARADIGM: POPULATION VALIDITY, THE SEX DIFFERENCE, STIMULUS CLARITY, AND CONTEXTUAL INFORMALITY. James P. O'Brien, Tidewater Community College, Virginia Beach VA 23453. This pilot study replicates Asch's (1951, 1956) independence-conformity paradigm with modifications to identify topics favorable for fuller empirical exploration. Initial analyses (i.e., stimulus clarity/ambiguity) were reported at the Academy's annual meeting by Schwabenbauer, Schwabenbauer, Larkin & O'Brien in 1999. Further analyses indicate a number of unresolved issues: (1) With samples more representative of the adult Americans, evidence for population validity is weak; (2) Contrary to the literature, there is scant evidence for a significant sex difference in independence-conformity; (3) Contextual informality (i.e., peer experimenters) merits further study; (4) Since the "Asch dilemma" is predicated on stimulus clarity, the finding of stimulus ambiguity in control treatments for both men and women supersedes all other factors

in importance. Since independent control replications are infrequent in the literature, especially for female baselines, systematic investigation of stimulus clarity in Asch-type paradigms is urgently needed before other issues can be adequately tested and resolved.

SPATIAL NAVIGATION AND LATENT LEARNING IN FEMALE BETTA SPLENDENS USING FOOD REINFORCEMENT. Casey Beatley, Raluca Brand & Andrew Velkey, Department of Psychology, Christopher Newport University, Newport News VA 23606. Betta splendens are exploratory predators within their native habitat. However, male Betta are residential and search for prey within a territory, whereas female Betta are non-residential and explore larger areas in search of potential mates and prey. In order to locate and identify a suitable mate, female Betta must navigate male territories while also foraging for prey. Latent learning occurs when changes in performance are not immediately observable after experience. Are Betta capable of latent learning, and do females learn their explored environments even when prey or mates are not encountered? In the present study, a single subject design with multiple replicates, 6 female Betta were placed in a complex maze containing two choice points and multiple alleys. Three of the subjects received food reinforcement for maze completion on every trial. The other 3 fish explored the maze freely for a period of 8' during each of the first 17 trials. The free-roaming fish later received reinforcement on trial 18. Results showed that the roaming fish demonstrated stability in maze completion more quickly than always-rewarded fish. While the present study revealed evidence of latent learning, excessive variability masked the effect. Future research should utilize a simpler maze to better demonstrate this effect.

DEVELOPMENT OF A COMPUTERIZED IMAGE ACQUISITION SYSTEM FOR ANALYSIS OF BETTA SPLENDENS BUBBLE-NESTING BEHAVIOR. Brand<sup>1</sup>, Casey Beatley<sup>1</sup>, Christine Searles<sup>1</sup>, Brian Roller<sup>2</sup>, & Andrew Velkey<sup>1</sup>, Dept. of Psych., Christopher Newport University, Newport News VA 23606 and <sup>2</sup>Dept. of Psych., The University of Arizona, Tucson AZ 85721. The present study demonstrates a computerized method for quantifying bubble nest size in male Betta splendens. Bubble nesting is a reproductively relevant behavior in Betta splendens. Certain environmental variables may influence bubble-nesting behavior, and investigators may wish to examine more closely the factors related to the presence and quality of bubble nests. In order to further study factors associated with bubble nesting, the development of a reliable methodology to measure various parameters of bubble nests is necessary. Several improvements to the procedure and apparatus were implemented in the current methodology in order to further increase the accuracy of image analysis. One major improvement in image acquisition results from using new equipment such as a Canon EOS 50D SLR Digital Camera. Images are captured using a RAW lossless format at 15.1 megapixels resolution. Higher resolution image acquisition results in better image analysis. Furthermore, a polarized filter was used in order to reduce glare. The current experiment aims to reduce measurement error that previously resulted from similarities in color intensity between bubbles and objects reflected due to glare. Results are discussed in regards to opportunities for refinement of measurement technique and application of the newly improved methodology.

THE RELATIONSHIP BETWEEN BIRTH ORDER AND IPIP BIG-FIVE FACTOR MARKERS. Laura A. Boettcher & Gayle T. Dow, Ph.D. Dept. of Psychology, Christopher Newport University, Newport News, VA, 23606. The purpose of this project is to investigate how one's birth order (only, youngest, middle, and oldest) potentially impacts scores of the IPIP Big-Five Factor Markers. Traits such as extraversion, agreeableness and neuroticism can be swayed by birth order. Birth order researchers have found that common traits exist within only, oldest, middle, & youngest born individuals, specifically first-borns tend to be more conscientious, whereas laterborns tend to be more agreeable. An online survey was completed by 192 participants with questions regarding participants' birth order and 50 questions from the IPIP. The IPIP Personality measure considers questions using the Big 5 personality model-extraversion, agreeableness, neuroticism, openness & conscientiousness. It was hypothesized that the youngest-borns would score higher on the agreeableness and openness. Trends emerged for extraversion and neuroticism.

STIMULUS DISCRIMINATION DURING AN INSTRUMENTAL LEARNING TASK IN DANIO RERIO. Morgan A. Cote-Coble & Christina Philyaw, Dept. of Psychology, Christopher Newport University, Newport News VA 23606. Using an instrumental choice procedure, 12 zebrafish (Danio rerio) were tested for their ability to discriminate between a stimulus associated with food reward (S+) and a stimulus associated with no food reward (S-). The subjects swam to the end of a T-maze and made a choice between two distinctly colored arms. Two squads of 6 fish were tested. For 6 of the subjects, the blue arm served as the S+ and was followed by a single bloodworm, while the green arm served as the S-. For the other 6 fish, the blue stimulus served as the S- and the green stimulus served as the S+. The right or left position of each S+ was randomly determined at the beginning of each trial. Subjects completed 3 trials per day until choice stabilization was evident, in which the same stimulus (S+ or S-) was selected in 8 out of 10 trials. Overall, 6 of the 12 subjects preferred the S+ over the S- while 2 fish failed to stabilize on a single reward; trials with the other four fish are nearing completion with most of them trending towards preference for the S+. Replicate trials are currently being conducted using another squad of naive subjects. The preference for signaled reward indicates that Danio rerio can be used for instrumental choice research, and future studies should use an inbred strain of Danio rerio to reduce genetic variability that may contribute to individual differences in the acquisition of instrumental responding.

# **Statistics**

SUPPORT VECTOR MACHINES WITH THE RAMP LOSS AND THE HARD MARGIN LOSS. J.P. Brooks, Dept. of Stat. Sci. and O.R., Virginia Commonwealth University, Richmond, VA 23284. The support vector machine (SVM) is a well-established method for classification based on an approach that emphasizes minimizing misclassification error while maximizing the distance between sets of correctly

classified observations. In training models, SVM uses a measure of error that is based on the Euclidean distance of observations from the separating surface. In the interest of increasing the robustness of SVM, we present two new integer programming formulations that incorporate the ramp loss and the hard margin loss, respectively. These formulations are able to accommodate nonlinear kernel functions that have made traditional SVM popular. The consistency of SVM with these loss functions is established. Analysis of simulated and real-world data sets indicates that Ramp Loss SVM is preferred over both Hard Margin Loss SVM and the traditional Hinge Loss SVM in the presence of outliers when a low-rank kernel function is employed.

STATISTICAL SIGNIFICANCE IN **EVALUATING SUPERSATURATED** DESIGNS. David J. Edwards, Dept. of Statistical Sciences and Operations Research. Virginia Commonwealth University, Richmond, VA 23284 & Robert W. Mee, Dept. of Statistics, Operations, and Management Science, Univ. of Tennessee, Knoxville, TN 37996. Two-level supersaturated designs (SSDs) are designs that examine more than n-1 factors in n runs. Although literature involving the construction of SSDs is plentiful, less has been written about analysis of data from these designs. Perhaps this is due in large part to the dearth of actual applications. Whether using forward selection or all-subsets regression, it is easy to select models from SSDs that explain a very large percentage of the total variation. Hence, naïve p-values can persuade the user that included factors are indeed active. We propose the use of a global model randomization test in conjunction with all-subsets to more appropriately select candidate models of interest. For settings where the number of factors is too large for repeated use of all-subsets to be applied repeatedly, we propose a short-cut approximation for the p-values based on the beta distribution. Finally, we propose a randomization test for reducing the number of terms in candidate models with small global p-values.

**SIMULATION OPTIMIZATION** USING TO CONSTRUCT **EFFICIENT** SCREENING STRATEGIES FOR CERVICAL CANCER. Laura A. McLay & Chris Foufoulides, Dept. of Stats. & Oper. Res., Virginia Commonwealth Univ. Cervical cancer is the second most common type of cancer in women worldwide. Because cervical cancer is usually asymptomatic until the disease is in its advanced stages, cervical screening is of central importance towards combating cervical cancer. Alternative screening strategies are evaluated from an economic point of view through In the literature, however, studies perform costcost-effectiveness analysis. effectiveness analysis on a limited number of de facto screening policies. At present, no attempt has been made to construct efficient screening strategies through optimization, before cost-effectiveness analysis is applied. In this study simulation optimization is used to construct efficient screening strategies for cervical cancer by properly timing the screenings. The constructed strategies are highly cost-effective when a small number of lifetime screenings is available, and are more cost-effective than screening strategies used in practice or considered in the literature so far, indicating the value of optimal timing for other screened diseases as well.

EVALUATING THE ASYMPTOTIC LIMITS OF THE DELETE-A-GROUP JACKKNIFE FOR MODEL ANALYSES. Phillip S. Kott, National Agricultural Statistics Service, Department of Agriculture, Fairfax VA 22030 & Steven T. Garren, Department of Mathematics and Statistics, James Madison University, Harrisonburg VA 22807. The delete-a-group jackknife can be e ectively used when estimating the variances of statistics based on a large sample. The theory supporting its use is asymptotic, however. Consequently, analysts have questioned its e ectiveness when estimating parameters for a small domain computed using only a fraction of the large sample at hand. We investigate this issue empirically by focusing on heavily poststrati ed estimators for a population mean and a simple regression coe cient, where the poststrati cation take place at the full-sample level. Samples are chosen using di erentially-weighted Poisson sampling. The bias and stability of delete-a-group jackknife employing either 15 of 30 replicates are evaluated and compared with the behavior of linearization variance estimators.

INFORMATION REDUCTION FOR BIAS AND VARIANCE ESTIMATION. Leonard A. Stefanski, Dept. of Stat., N.C. State Univ., Raleigh, NC 27696-8203. The jackknife and bootstrap are two well-known methods of reducing bias and estimating variance. Simulation-extrapolation is a method of reducing bias and estimating variance in measurement error models that works by adding more error to the observed data. Omitting an observation (jackknife), sampling from the observed data (bootstrap), and adding noise to data (simulation-extrapolation) are all ways of reducing information in a data set. In this talk I show that all three methods are conceptually similar when viewed in terms of information reduction, and argue that doing so is sometimes advantageous.

# Structural Biology, Biochemistry and Biophysics

NEUROSTEROID REGULATION OF IONOTROPIC GLUTAMATE RECEPTORS. Sarah Rhoads & Lisa Gentile, University of Richmond. AMPA, NMDA and kainate receptors belong to the ionotropic glutamate receptor (iGluR) family. As binding to glutamate, a major fast excitatory neurotransmitter, causes activation of these channels, they play an important role in synaptic plasticity, memory and learning. Our research focuses on understanding how these receptors are regulated binding for potential applications in conditions such as Alzheimer's and Parkinson's disease. The data presented here is aimed at understanding the differential regulation of NMDA receptors by the endogenous neurosteroids pregnenolone sulfate (PS) and 3α-hydroxy-5βpregnan-20-one sulfate (PREGAS). PS potentiates the activity of NMDA receptors containing an NR2B subunit while it inhibits those containing an NR2D subunit. PREGAS negatively regulates all iGluRs. Intrinsic and extrinsic fluorescence studies will be presented that confirm the binding of the NMDA NR2B S1S2 and amino terminal domain (ATD) to both PS and PREGAS. Unlike the NR2B subunit, the NR2D S1S2 domain does not bind to PS and PREGAS, however the NR2D ATD does bind to both neurosteroids. Data from isothermal titration calorimetry and Stern-Volmer

analysis will be presented to help differentiate the binding site of each of these neurosteroids on both the NMDA NR2B and NR2D subunits.

CHARACTERIZATION OF RECOMBINANT ASPERGILLUS FUMIGATUS SIDA: FLAVIN-DEPENDENT N-HYDROXYLASE WITH COFACTOR. Samuel W. Chocklett & Pablo Sobrado Department of Biochemistry, Virginia Tech, Blacksburg, VA 24060. Aspergullus fumigatus (Af) SidA, is the flavindependent enzyme that catalysis the NADPH-dependent hydroxylation of L-ornithine in ferrichrome biosynthesis. Af SidA was recombinantly expressed and purified as a soluble tetramer with a bound FAD cofactor. Af SidA is the first member of this class of flavin monoxygenases to be isolated with a tightly bound flavin cofactor. The enzyme showed typical saturation kinetics with respect to L-ornithine, while substrate inhibition was observed at high concentrations of reduced coenzyme. Increasing concentrations of hydrogen peroxide were measured as a function of coenzyme concentration, indicating that inhibition was caused by an increase in uncoupling. Af SidA is highly specific for its amino acid substrate, only hydroxylating L-ornithine. In contrast, an 8-fold preference in the catalytic efficiency was determined for NADPH as compared to NADH. In the absence of substrate, Af SidA can be reduced by NADPH and a stable C4a-(hydro)peroxyflavin intermediate is observed. The decay of this intermediate is accelerated by L-ornithine binding, and was only stabilized by NADPH and not by NADH, suggesting a role for NADP+ in the stabilization of intermediates in the reaction of Af SidA. NADP is a competitive inhibitor with respect to NADPH, demonstrating that Af SidA forms a ternary complex with NADP<sup>+</sup> and Lornithine for catalysis. These data indicates that Af SidA likely proceeds by a sequential kinetic mechanism. Supported in part by the Allan T. Gwathmey Chemistry award from the Virginia Academy of Sciences and Ralph Powe award from ORAU.

ADVANCING THERAPEUTICS FOR ALZHEIMER'S DISEASE MOLECULAR DYNAMICS SIMULATIONS. Justin A. Lemkul & David R. Bevan, Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Neuronal deposition of the amyloid β-peptide (Aβ) is believed to trigger the symptoms of Alzheimer's disease, the leading cause of senile dementia that afflicts over 5 million Americans. In vitro and in vivo studies suggest that natural products, such as flavonoids, may be effective in preventing and reversing this protein aggregation, but their mechanism of action is unknown. We conducted molecular dynamics (MD) simulations on a model of the AB protofibril in the presence of the flavonoid morin to understand how this compound, one of the most potent antiaggregation flavonoids, may function in destabilizing pre-formed Aβ aggregates. Our results indicate that morin principally binds to the end of the protofibril, occupying backbone hydrogen bonds that are exposed to solvent and would otherwise be accessible to an incoming peptide. We call this binding mode a "capping network," and we have demonstrated that this configuration effectively blocks the attachment of an incoming peptide. Morin can also penetrate into the hydrophobic core of the protofibril structure, where it associates with the Asp23-Lys28 salt bridges and interferes with backbone hydrogen bonding to destabilize the native structure. The material is based

upon work supported by the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) of the National Science Foundation under Agreement No. DGE-0333378, and by the Institute for Critical Technology and Applied Science (ICTAS) at Virginia Tech.

# VIRGINIA JUNIOR ACADEMY OF SCIENCE MAY 21, 2010 69th ANNUAL MEETING AWARDS JAMES MADISON UNIVERSITY HARRISONBURG, VA

## AGRICULTURE AND ANIMAL SCIENCE

Honorable Mention: ROHAN MATHEW

George H. Moody Middle School

Honorable Mention: CHANNING A. MOSS AND SAMANTHA N.

PREASEAU

Chesapeake Bay Governor's School

Honorable Mention: KAYLA E. PFAB

Deep Run High School

Third Place: KRYSTA R. TROUT

Shenandoah Valley Governor's School

Second Place: MATTHEW T. KING

George H. Moody Middle School

First Place: CLAYTON M. GEIPEL

Mills E. Godwin High School

# ANIMAL BEHAVIOR (ETHOLOGY)

Honorable Mention: ANATEVKA S. RIBEIRO

Mountain Vista Governor's School

Honorable Mention: GRAYSON D. SANNER

H.B. Woodlawn

Third Place: RACHEL M. BOAG

Mountain Vista Governor's School

Second Place: PRIYA CHITTUR, CYNTHIA LI AND

DIVYA BATHEY

Thomas Jefferson High School for Science and

Technology

DANIEL JANG, ARMAN KHOJANDI AND

**GOVIND MATTAY** 

Thomas Jefferson High School for Science and

Technology

**BOTANY A** 

Honorable Mention:

MALLORY J. BANTON

Hanover High School

Honorable Mention:

ANANT KHARKAR

George H. Moody Middle School

Honorable Mention:

NAVEEN C. KOTHA

George H. Moody Middle School

Third Place:

HANNAH M. GOOD

Deep Run High School

Second Place:

MADHURA V. CHITNAVIS

Roanoke Valley Governor's School

First Place:

HARTZEL E. GILLESPIE

Central Virginia Governor's School

**BOTANY B** 

Honorable Mention:

TYLER A. LAREDO

Kenmore Middle School

Honorable Mention:

CHRISTOPHER M. NOWAK

Douglas Freeman High School

Honorable Mention:

IMANI B. TINTER

Washington-Lee High School

Third Place:

MAVRA MASOOD

George H. Moody Middle School

Second Place:

BIYUAN ZHAO

Southwest Virginia Governor's School

ERIK G. ZORN

Roanoke Valley Governor's School

# CHEMISTRY A

Honorable Mention:

JOOHEE CHOI

Langley High School

Honorable Mention:

EMILY E. COOK

Washington-Lee High School

Honorable Mention:

PRASANNA G. JOSHI

Mills E. Godwin High School

Third Place:

VIJAY GOVINDARAJAN

Mills E. Godwin High School

Second Place:

SAUMIL BANDYOPADHYAY

Maggie L. Walker Governor's School

First Place:

CAITLIN A. CHERESNOWSKY AND

JESSICA K. KERSEY Gloucester High School

#### CHEMISTRY B

Honorable Mention:

PRIYA M. SARKAR

George H. Moody Middle School

Honorable Mention:

ISAAC M. VOGLER

Mountain Vista Governor's School

Third Place:

SAMEER M. SARKAR

Maggie L. Walker Governor's School

Second Place:

DONG JOO YOON AND HYE JOO YOON

Langley High School

First Place:

JEE IN SEO

Thomas Jefferson High School for Science and

Technology

#### COMPUTER SCIENCE

MATTHEW R. BONGIOVI Honorable Mention:

Mountain Vista Governor's School

LAURENCE A. JONES Honorable Mention:

Atlee High School

DEREK W. LOFTIS Honorable Mention:

Ocean Lakes High School

Third Place: MATTHEW H. MARTIN

Ocean Lakes High School

Second Place: MATTHEW A. FRAZIER

Mountain Vista Governor's School

First Place: JAMES J. WANDA

Washington-Lee High School

# CONSUMER SCIENCE A

Honorable Mention: ANNIE B. BLEMKER AND MORGOT C.

HAIHALL

Robious Middle School

Honorable Mention: ARIEL L. HARPER

Shenandoah Valley Governor's School

JOHN W. BLOOR Honorable Mention:

Deep Run High School

Third Place: BENJAMIN M. BROOKS

Shenandoah Valley Governor's School

KATHRYN E. BAXLEY Second Place:

Shenandoah Valley Governor's School

First Place: ALISON E. EDDINS

George H. Moody Middle School

## CONSUMER SCIENCE B

Honorable Mention: MEGAN R. LOWERY

Shenandoah Valley Governor's School

Honorable Mention: LUCY C. PLANT

Shenandoah Valley Governor's School

Honorable Mention: MEGAN E. WINE AND KATHERINE H.

MONKS

Robious Middle School

Third Place: HUNTER J. MICHAEL

Shenandoah Valley Governor's School

Second Place: STEFAN MOSCALU

Central Virginia Governor's School

First Place: CARAHLINE M. STARK

Southwest Virginia Governor's School

#### EARTH AND SPACE SCIENCE

Honorable Mention: UTKARSHA BHAVE

George H. Moody Middle School

Honorable Mention: KATHARINE E. HINES

George H. Moody Middle School

Honorable Mention: JUSTIN W. LAU

George H. Moody Middle School

Third Place: GEORGE M. LUPTON, IV

Central Virginia Governor's School

Second Place: PAUL J. ZIVICK

Central Virginia Governor's School

First Place: BRANDON T. KATONA

Mills E. Godwin High School

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# ENGINEERING A

Honorable Mention: SETH C. AUSTIN

Shenandoah Valley Governor's School

Honorable Mention: JONATHAN KIM AND VANCE P. BARDEN

Deep Run High School

Honorable Mention: ROBERT J. KUCZMARSKI

Roanoke Valley Governor's School

Third Place: WILLIAM E. KUNKEL

George H. Moody Middle School

Second Place: PAUL J. DIMARTINO

George H. Moody Middle School

First Place: LAUREN E. JOHNSON

Mills E. Godwin High School

ENGINEERING B

Honorable Mention MANHAS NARRA

Thomas Jefferson High School for Science and

Technology

Honorable Mention: DOMENICK V. PUZIO

Shenandoah Valley Governor's School

Honorable Mention: JOSEPH W. WOOD

Shenandoah Valley Governor's School

Third Place: CATESBY K. WOLSKI

George H. Moody Middle School

Second Place: JAMES P. STEWART

Roanoke Valley Governor's School

First Place: JAMES C. TYLER

George H. Moody Middle School

## ENVIRONMENTAL SCIENCE A

Honorable Mention: REBEKAH W. BARKER

Deep Run High School

Honorable Mention: MEREDITH A. BOWER

Tandem Friends School

Honorable Mention: JEREMY BURKE

Central Virginia Governor's School

Third Place: KELSI BAUGHAN, HANNAH GILL AND

CAITLIN TIGNOR

Chesapeake Bay Governor's School

Second Place: LEAH J. COATES

Chesapeake Bay Governor's School

First Place: NICOLE C. BOYD AND CHRISTINA S.

MOORE

Thomas Jefferson High School for Science and

Technology

## ENVIRONMENTAL SCIENCE B

Honorable Mention: BRANDON E. COOPER

Chesapeake Bay Governor's School

Honorable Mention: MEAGAN C. DAVID

Bishop Denis J. O'Connell High School

Honorable Mention: AMBER S. FAUBER

Chesapeake Bay Governor's School

Third Place: JANAI A. GOLDEN

George H. Moody Middle School

Second Place: ARIEL DEUTSCH, HANNAH LAN AND

PRIYA PATEL

Thomas Jefferson High School for Science and

Technology

First Place: PATRICK S. HOWARD

Chesapeake Bay Governor's School

## ENVIRONMENTAL SCIENCE C

Honorable Mention: SARAH G. MURPHY

Douglas Freeman High School

Honorable Mention: GRACE A. PERKINS

Chesapeake Bay Governor's School

Third Place: RACHAEL L. LIPSCOMB

Chesapeake Bay Governor's School

Second Place: MATTHEW F. MANN

Chesapeake Bay Governor's School

First Place: GAVIN T. KLINE

Central Virginia Governor's School

#### ENVIRONMENTAL SCIENCE D

Honorable Mention: KATE E. TILLOTSON

Central Virginia Governor's School

Honorable Mention: AKSHAR WUNNAVA

Thomas Jefferson High School for Science and

Technology

Third Place: JOHN R. RYAN

Oak Knoll Middle School

Second Place: EMMA RODVIEN

Yorktown High School

First Place: CHRISTINE E. WILLIAMS

Chesapeake Bay Governor's School

## GENETICS AND CELLULAR BIOLOGY

Honorable Mention: CHRISTOPHER A. HOLLINGSWORTH

West Potomac High School

# **VJAS AWARDS**

Honorable Mention: JILL E. JACKSON

Shenandoah Valley Governor's School

Honorable Mention: ALYSSA N. OSIMANI

Blacksburg High School

Third Place: GALEN E. NIXON

George H. Moody Middle School

Second Place: CLAIRE L. COOPER

Thomas Jefferson High School for Science and

Technology

First Place: NADINE NIKOLOVA

Deep Run High School

**MATHEMATICS** 

Honorable Mention: STEVEN P. TRAYER

Mountain Vista Governor's School

Third Place: THOMAS R. STEIMEL

Mountain Vista Governor's School

Second Place: BRETT C. BERGER

Woodberry Forest School

First Place: HEMING ZHAO

Southwest Virginia Governor's School

MEDICINE AND HEALTH A

Honorable Mention: KARA M. ADAMS

George H. Moody Middle School

Honorable Mention: KYLIE J. ARMSTRONG

Shenandoah Valley Governor's School

Honorable Mention: SARA K. GARBER

Shenandoah Valley Governor's School

Third Place: SAYANTANEE DAS

George H. Moody Middle School

Second Place: GRANT S. BROUSSARD

George H. Moody Middle School

First Place: NATASHA N. CHOWDRY

Central Virginia Governor's School

# MEDICINE AND HEALTH B

Honorable Mention: SE WOONG JEONG

Roanoke Valley Governor's School

Honorable Mention: MANON LOUSTAUNAU

Washington-Lee High School

Honorable Mention: TERESA PADGETT

Central Virginia Governor's School

Third Place: ELAINE M. HARRINGTON

Bishop Denis J. O'Connell High School

Second Place: JESSICA W. LAU

Mills E. Godwin High School

First Place: SAMANTHA M. MARQUEZ

Robious Middle School

## MEDICINE AND HEALTH C

Honorable Mention: WHITNEY N. ROSSER

Central Virginia Governor's School

Honorable Mention: ANNESHA SENGUPTA

Manchester Middle School

Honorable Mention: ROHAN SHARMA

George H. Moody Middle School

Third Place: DAVID R. SHORE

Maggie L. Walker Governor's School

Second Place: ZEB ZAHEER

Yorktown High School

ASHLEY R. TAYLOR

Southwest Virginia Governor's School

# MICROBIOLOGY A

Honorable Mention:

CLAIRE A. BOBST Yorktown High School

Honorable Mention:

ELIZABETH T. GIBSON AND NICOLE C.

BRODER

H.B. Woodlawn

Honorable Mention:

MARK T. SCHREIBER Wakefield High School

Third Place:

SACHITH GULLAPALLI

Roanoke Valley Governor's School

Second Place:

CARLSON M. SAWYER

New Horizons Governor's School

First Place:

KATHRYNLYNN WELLS JENKINS

York High School

# PHYSICAL SCIENCE

Honorable Mention:

HANNAH FITZMAURICE Swanson Middle School

Honorable Mention:

CAMERON D. GIBSON

Stonewall Jackson Middle School

Honorable Mention:

CONNOR HENNESSEY-NILAND

Swanson Middle School

Third Place:

ARJUN K. JAINI

Stonewall Jackson Middle School

Second Place:

ISABELLE S. STERN

George H. Moody Middle School

THOMAS N. TOMBES AND JEREMIAH W.

HOYT

Robious Middle School

**PHYSICS** 

Honorable Mention:

MADISON A. HAMBLEN

Deep Run High School

Honorable Mention:

NICHOLAS K. PETERMAN

Deep Run High School

Honorable Mention:

HENRY M. TESSIER

Swanson Middle School

Third Place:

CALEB JONES

Gildersleeve Middle School

Second Place:

BENJAMIN L. ANDERSON

Patrick Henry High School

First Place:

ZACHARY TERNER AND MENJAMIN

TERNER

Mills E. Godwin High School

PSYCHOLOGY - GENERAL

Honorable Mention:

KIRBY J. CLARK

Washington-Lee High School

Honorable Mention:

RACHEL M. DRESCHER

Mountain Vista Governor's School

Honorable Mention:

ANDREW W. DUDKA

Washington-Lee High School

Third Place:

ELIZABETH P. BELL

George H. Moody Middle School

Second Place:

DAVIS A. KLABO

Deep Run High School

GRACE E. BENSON Blacksburg High School

# PSYCHOLOGY - LEARNING & PERCEPTION A

Honorable Mention:

AMANDA J. HINCKLE

Deep Run High School

Honorable Mention:

MALLORY E. MCKENZIE

Washington-Lee High School

Honorable Mention:

ISABEL E. AMEND

Washington-Lee High School

Third Place:

CHARLES H. HALE

Chesapeake Bay Governor's School

Second Place:

PAUL B. GIRGIS

George H. Moody Middle School

First Place:

JOHN J. BOYLE, III AND TARA KENNEDY

Deep Run High School

#### PSYCHOLOGY - LEARNING & PERCEPTION B

Honorable Mention:

ADAM T. PHILLIPS

Deep Run High School

Honorable Mention:

SRISHTI SANYA

George H. Moody Middle School

Honorable Mention:

SAMANTHA F. SPYTEK

Wakefield High School

Third Place:

HOLLY M. SEALEY AND MEGHAN E.

WEEDON

Deep Run High School

Second Place:

SONIA PHENE

Washington-Lee High School

First Place: ADAM D. RELLICK

Chesapeake Bay Governor's School

## **PSYCHOLOGY - SOCIAL**

Honorable Mention: TAYLOR K. CHAMNESS

Bishop Denis J. O'Connell High School

Honorable Mention: RYAN A. OPPENHEIM

Robious Middle School

Honorable Mention: RACHEL E. SCHWARTZ

Washington-Lee High School

Third Place: BENJAMIN P. MCGARY

Ocean Lakes High School

Second Place: LAKSHMI M. BODAPATI

George H. Moody Middle School

First Place: JASMINE P. JONES

Central Virginia Governor's School

STATISTICS

Honorable Mention: KATHERINE P. HUGHES

Shenandoah Valley Governor's School

Honorable Mention:

CHAMBON

WILLIAM J. LUXHOJ AND JACOB L.

Deep Run High School

Honorable Mention: VICTORIA K. AMATO

Shenandoah Valley Governor's School

Third Place: CORY B. BRANT

Shenandoah Valley Governor's School

Second Place: ZACHARY W. BARLOW

Shenandoah Valley Governor's School

First Place: MEGAN L. MOHR

Yorktown High School

ZOOLOGY

Honorable Mention: CHRISTOPHER S. EISENMAN

Mountain Vista Governor's School

Honorable Mention: ANDREW T. LEFFLER

George H. Moody Middle School

Honorable Mention: KELLY C. SCHUMANN

Thomas Jefferson High School for Science and

Technology

Third Place: BRADY K. BROWN

George H. Moody Middle School

Second Place: MARY D. SUN AND SHAWN C. TSUTSUI

Thomas Jefferson High School for Science and

Technology

First Place: AMEYA A. CHUMBLE

Galileo Magnet High School

# Special Awards

Botany Section Award, given by the Botany Section of the VAS, to the best paper on a botanical subject.

ERIK G. ZORN

Roanoke Valley Governor's School

Mathematics Award for the paper that evidences the most significant contribution in the field of Mathematics.

HEMING ZHAO

Southwest Virginia Governor's School

Statistics Award for the paper that evidences the most significant contribution in the field of Statistics.

MEGAN MOHR

Yorktown High School

Roscoe Hughes Award for the best paper in the field of Genetics.

**GALEN NIXON** George H. Moody Middle School

Rodney C. Berry Chemistry Award for the paper that evidences the most significant contribution in the field of chemistry.

> JEE IN SEO Thomas Jefferson High School for Science and Technology

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry will be given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life.

> SAMEER SARKAR Maggie L. Walker Governor's School

Catesby Jones - Russell J. Rowlett Award for the Best Research Paper of the Year.

> NICOLE C. BOYD AND CHRISTINA S. MOORE Thomas Jefferson High School for Science and Technology

Virginia Sea Grant College Program Award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research.

> GRACE A. PERKINS Chesapeake Bay Governor's School

American Cancer Society Award - This award is to recognize outstanding science papers related to cancer research. These awards are funded by the American Cancer Society (Virginia Council).

Second Place

SAMEER SARKAR Maggie L. Walker Governor's School

First Place

SAYANTANEE DAS George H. Moody Middle School

The Gamma Sigma Delta Award (Agriculture). Presented by the VPI & SU Chapter of the Honor Society of Agriculture. This award is presented technologies and/or concepts in agriculture forestry, or veterinary medicine.

CLAYTON GEIPEL
Mills E. Godwin High School

Dominion - W.W. Berry Award. This award is given by Dominion Virginia Power in honor of Mr. W. W. Berry who was a past Chairman of the Board of VA Power.

JAMES C. TYLER George H. Moody Middle School

The Joyce K. Peterson Award is presented for the outstanding paper by a middle school student. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools.

SAYANTANEE DAS George H. Moody Middle School

The Ann M. Hancock Award - This award is given to the best paper in cellular biology and is given in memory of Anne Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee.

NANDINE NIKOLOVA
Deep Run High School

VABE Award - This award is presented by the Virginia Association of Biology Educators and is given for outstanding research in the Zoology section.

AMEYA CHUMBLE Galileo Magnet High School

Virginia Museum of Natural History Award - Presented by the Friends of the Virginia Museum of Natural History in recognition of significant contribution in the study and interpretation of Virginia's Natural Heritage.

> MATTHEW MANN Chesapeake Bay Governor's School

Trip to AJAS - AAAS Meeting for two students for presenting outstanding papers.

Winner: DANIEL JANAG, ARMAN KHOJANDI AND GOVIND MATTAY

Thomas Jefferson High School for Science and Technology

Honorary Membership - AAAS given to two students.

SUCHANA COSTA
Washington-Lee High School

WILLIAM NOSTRA Atlee High School

Honorary Membership - VAS given to a student.

SE JEONG Roanoke Valley Governor's School

Bethel High School Scholarship - This \$1,000 Scholarship Award comes from the interest earned from a \$10,000 endowment contributed by the students of Bethel High School, Hampton, Va., over a two year period. This award is based on both the students presentation and paper.

CHRISTINE WILLIAMS
Chesapeake Bay Governor's School

Henry MacKenzie Environmental Scholarship - This \$5,000 scholarship will be awarded to the student whose paper evidences the most significant contribution in the field of Environmental Science dealing with the James River Basin and Chesapeake Bay. The Virginia Endowment and VJAS offer this scholarship in tribute to the outstanding and generous services of Judge Henry W. MacKenzie, Jr., one of the founding directors who has a great interest in the James River and the Chesapeake Bay.

LEAH COATES
Chesapeake Bay Governor's School

Frances and Sydney Lewis Environmental Scholarship: A \$14,000 scholarship (\$3,500 per year for four years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This scholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental Endowment.

CHRISTINE WILLIAMS
Chesapeake Bay Governor's School

E.C.L. Miller Science Teacher of the Year Award is given to an outstanding science teacher. An all-expense-paid trip to the next VAST meeting held in November.

HEATHER GREEN
Ocean Lakes High School

## New officers:

Secretary - Luke Faraone Co- President - Ana O'Harrow Co-President - William Nostra

Regional Directors- Alan Booth (II)

Mannas Narra (IV)

# Ivey F. Lewis Distinguished Service Award



Presenting Award, VAS President D'Arcy P. Mays, Accepting Award for, J. Sargeant Reynolds Community College, John N. Ambrose, Associate Vice President of Technology.

# J.Sargeant Reynolds Community College May 2009

J.Sargeant Reynolds Community College was awarded the Virginia Academy of Science Ivey F. Lewis Distinguished Service Award. Since the very beginning of J. Sargeant Reynolds Community College in 1972, it has supported the Academy by encouraging its faculty to become members, supporting the Visiting Scientists program and supporting travel to the annual meetings. Even during hard economic times of budget cutbacks, it supported faculty travel to Council and annual meetings. One Academy member was in 35 years, never refused travel support. JSRCC supported the Virginia Journal of Science by supporting its editor and allowing the Journal to be managed and typeset in the Science Department. The College supplied and maintained the computer, printer, scanner and programs needed to produce the Journal. In fact, the very first scanner purchased by the College was stationed in the editor's office. Correspondence with authors was also supported by the college. The college has also allowed the Academy to hold Council and committee meetings in its classrooms.

When J. Sargeant Reynolds Community College developed its initial web presence, it encouraged Dr. Jim Martin to learn HTML and produce a personal web page, and within a year allowed him to develop a prototype academy web page on its server. By mid 1997, the Academy had its own URL and a fully operational web site, supported by the College. To this day, the College graciously houses the Academy server.

J. Sargeant Reynolds Community College has in the past and is continuing to make significant contributions to the Virginia Academy of Science.



Lisa L. Martin

Lisa L. Martin was awarded the Virginia Academy of Science Ivey F. Lewis Distinguished Service Award. Lisa became increasingly familiar with the Academy and its operation as she attended academy meetings for the past 38 years. Twenty four years ago she began working with the Virginia Junior Academy of Science when she took on a small part-time job in the Academy office offered by Dean Decker, the Junior Academy director. She advanced the computerization of the Junior Academy, edited, typeset and produced the Proceedings of the Virginia Junior Academy of Science, maintained the records, correspondence, and management of the office during a time of increasing complexity as the Junior Academy grew in size to over 1200 students. Working with the successive Junior Academy Directors (Dean Decker, Don Cottingham and Susan Booth) Lisa attended every Academy meeting, many times working past midnight to produce the hundreds of winner certificates and the script for the next morning's Junior Academy Awards assembly. She produced the first digital copy (a CD) of the Proceedings for distribution, greatly reducing the Proceeding's cost to the Academy.

During this time in the Academy office, Lisa became increasingly involved with the operation of the Senior Academy She worked closely with Executive Secretaries Blanton Brunner and Arthur Burke, and Executive Officer Jerry Bass. She was the main driving force bringing the Academy Office into the computer age. She computerized and maintained the membership and dues records, managed purchase and service of equipment, office and storage facilities. Lisa has become the corporate memory of the Academy and has contributed significantly to the Virginia Academy of Science.

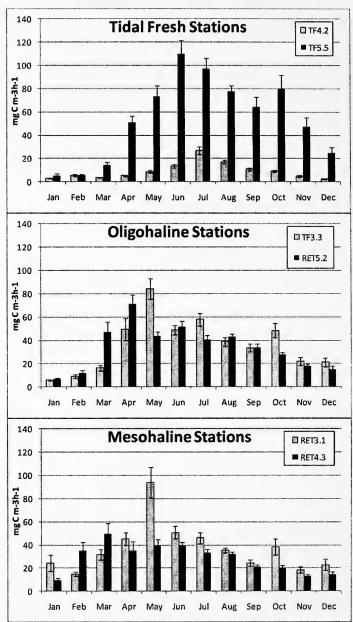
# Corrections to the paper "Phytoplankton productivity in the tidal regions of four Chesapeake Bay (USA) tributaries". Virginia Journal of Science. 2007 58(4):191-204, by K.K. Nesius, H.G. Marshall, and T.A. Egerton.

During a recent re-analysis of data we have identified necessary corrections associated with the productivity rates given in this paper. These include values for stations originally presented in the article's Table 2 and Figures 3-5 which are amended and given below, with the adjusted mean annual productivity rates for these stations ranging from 48 to 193 g C m<sup>-2</sup> yr<sup>-1</sup>. The long-term trends accompanying this reanalysis indicate increased productivity occurring at stations within these tributaries. These trends will be addressed in a future publication with data over an expanded two decade-plus time period evaluated. We would refer the reader to contact the authors with any questions regarding these corrections.

K.K. Nesius, H.G. Marshall and T.A. Egerton Department of Biological Sciences Old Dominion University Norfolk, Virginia 23529-0266

TABLE 2. Annual range and averages of river productivity rates from stations from 1989-2001. Thidal freshwater (TF), Oligohaline (OLIG), Mesohaline (Mes).

		Range of annual productivity (mg C m-2 h-1)	Average annual productivity (mg C m02 h-1)
Rappahanno	ock River		
TF3.3	(Olig)	11.78 - 66.99	36.12
RET3.1	(Mes)	8.35 - 80.23	36.44
Average			36.28
York/Pamur	nkey Rivers		
TF4.2	(TF)	1.60 - 18.57	9.50
RET4.3	(Mes)	7.51 - 68.38	27.50
Average			18.50
James River	•		
TF5.5	(Olig)	12.81 - 99.34	53.86
RET5.2	(Mes)	13.10 - 57.71	34.14
Average			44.00



Figures 3-5. Monthly average productivity rates (mg C m<sup>-3</sup> h<sup>-1</sup>) for tidal freshwater, oligohaline and mesohaline stations 1989-2001.

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George, Kelly L		Katiyar, Neerja	
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# Foundational Checklist of the Amphibians of Wise County, Virginia

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#### ABSRTACT

The Appalachian Mountains are arguably home to the highest degree of amphibian diversity in the world, particularly caudate (salamander) biodiversity. Despite the high degree of amphibian endemism in the Appalachians, several regions remain unsurveyed for amphibian species. In addition to this knowledge gap, we are in the midst of alarming amphibian biodiversity loss. Thus, it is of the utmost importance to bridge this knowledge gap by conducting surveys before some of these amphibian species are lost. We surveyed Wise County (previously unsurveyed county in the Appalachian Mountains with no records existing in the primary literature) over two years to assess amphibian species presence. We found 23 different species of amphibians (eight species of frogs and toads; 15 species of salamanders). In addition, we report five new amphibian species occurrences previously unreported in the primary literature within Wise County. However, not all amphibian species expected to occur in Wise County were observed. The primary suspected reason for their lack of occurrence involves habitat loss and/or modification, since the region is heavily exploited for coal and lumber. Overall, our study provides invaluable data in current times of amphibian biodiversity concern as they clarify and expand our knowledge of known amphibian species within the area. Using our work as a foundation, future surveying could assess whether amphibian biodiversity of Wise County are experiencing growth, stability, or decline.

#### INTRODUCTION

In the wake of accelerated anthropogenic disturbances to nearly every ecosystem on the planet (Walker et al. 2005), population monitoring has never been more critical. Most scientists would argue that we are currently in the wake of the sixth major extinction in Earth's history (Wake and Vredenburg 2008). For example, approximately 33% of all extant amphibian species are facing declining populations (Stuart et al. 2004). Such a dramatic loss of amphibian biodiversity has been linked to several events, including habitat loss and disease (Kiesecker et al. 2001; Mendelson et al. 2006). While the majority of amphibian populations declines appear to be occurring in the tropics (Wake and Vredenburg 2008), amphibian species in the more temperate

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regions are not without their vulnerabilities. However, detecting population declines, while being vitally important, is not easily done – particularly in amphibians. Most amphibian populations are constantly in a state of natural flux (Pechmann and Wilbur 1994), and are highly dependent on uncontrollable environmental influences (such as food abundance, climate, and predation). Nevertheless, the preservation and conservation of amphibians is dependent upon population data. Unfortunately, such data are rarely seen in the primary literature; or if they are reported, it is likely because the population has already declined.

Concerning amphibian diversity in temperate North America, the Appalachian Mountains are arguably the most diverse – especially concerning caudate (salamander) diversity. The Appalachians are home to several endemic caudate species (see Lannoo 2005). Because of this high degree of endemism, monitoring populations over time is essential in conservation efforts of these rare and unique species. However, before extensive monitoring programs can be initiated, foundational groundwork concerning the list of native species must be documented. Herein, we report the first list (to the best of our knowledge) of amphibians occurring in Wise County, Virginia in the primary literature. There are, however, works that report the historic distribution of herpetofauna (amphibians and reptiles) in the Commonwealth (see Mitchell and Reay 1999). However, Mitchell and Reay (1999) explicitly state, on several occasions, that extensive fieldwork needs to be conducted in southwest Virginia (including Wise County) to further clarify the unknown distributions of several herpetofaunal species. The goal of this project was to answer this direct call.

#### **METHODS**

All amphibian species observations were within Wise County, Virginia (USA) borders during the summer and fall months of 2009, and spring, summer, and fall months of 2010. Wise County is located in southwest Virginia. It is bordered by Pike County, Kentucky (north), Dickenson County, Virginia (northeast), Russell County, Virginia (east), Scott County, Virginia (south), Lee County, Virginia (southwest), Harlan County, Kentucky (west), and Letcher County, Kentucky (northwest). Wise County covers approximately 1050 km², including approximately 3 km² of water coverage.

Wise County offers truly unique heterogeneous habitats all along elevation gradients since it is situated in the midst of the Appalachian Mountains. These habitats include coniferous, deciduous, and mixed forests and old fields, coupled with urbanized areas. We sought to survey all habitat types that would yield potential amphibian species observations. As a result, careful and thorough reference book and atlas reviews (e.g., Hulse et al. 2001; Lannoo 2005; Mitchell and Reay 1999; Petranka 1998) were conducted in order to identify preferred habitat types of all amphibian species historically known to occur within Wise County. Specifically, we surveyed both public and private land areas (with permission from land owner(s)). However, the vast majority of this study was conducted on public-use land. The bulk of our public-use land surveys were within various regions of the Jefferson National Forest. Indeed, a large potion of Wise County is part of the Jefferson National Forest. Specifically concerning the Jefferson National Forest, we surveyed the following areas and their immediate surroundings: Cave Springs Recreation Area (near Big Stone Gap, Virginia;

including its Loop Trail and numerous adjacent springs), Stone Mountain Trail (near Big Stone Gap, Virginia; including the numerous small streams and small waterfalls adjacent to the trail), High Knob Recreation Area (near Norton, Virginia; including its wooded Day-Use Areas, Lake Trail, Lake Loop Trail, and the numerous adjacent small streams to these trails), Flag Rock Recreation Area (near Norton, Virginia), Guest River Gorge Trail (near Coeburn, Virginia; including the Clinch River and its following branches: Jaybird, Crab Orchard, Pine Orchard, Hurricane, Flat, Lick Log, and Lick), Little Stony Falls (near Coeburn, Virginia; including the small streams along the Little Stony Falls Trail), Red Fox Trail (near Pound, Virginia; including its numerous small streams adjacent to the trail), Pound Lake (near Pound, Virginia; including its numerous small streams flowing in and out of the lake), Cane Patch Recreation Area (near Wise, Virginia; including areas surrounding/in Bad Creek and North Fork Creek), and Phillips Creek Recreation Area (near Wise, Virginia; including Phillips Creek Trail Loop and the numerous small creeks and waterfalls adjacent to the trail). In addition to these public-use localities within the Jefferson National Forest, we surveyed various public-use regions on the approximately 1.6 km<sup>2</sup> campus of the University of Virginia's College at Wise (including its wetlands, three ponds, numerous small streams, and the surrounding wooded area). Opportunistic amphibian species captures, such as chance encounters while driving to and/or from targeted field sites, accounted for a minute portion of our survey.

Most of our amphibian species observations were direct. When surveying terrestrial habitats, we captured amphibians by hand by lifting cover objects (natural cover objects, such as logs and rocks; or unnatural, such as mattresses and tires). The same strategy was employed for surveying most aquatic habitats, with cover objects on stream banks being lifted. However, in some instances, we used dip-nets in aquatic habitats that had a high degree of leaf litter compared to cover object availability along the bank margins. In some instances involving anuran (frog and toad) species, observations were indirect and accomplished by listening for species-specific calls. To further increase our observation success of targeted species, surveys were conducted at various times of the day/night – depending upon the exact ecological niche of each target species. All scientific and standard English names (common names) of each species follow Crother et al. (2000; 2003).

#### RESULTS

In total, we observed 23 amphibian species in Wise County, Virginia (see Table 1 for complete list). Specifically, we observed eight anuran (frogs and toads) and 15 caudate species. According to Mitchell and Reay (1999), we observed 18 of their 23 (approximately 78%) previously known amphibian species within the county (see Table 1). In addition, we report new occurrences of *Ambystoma maculatum* (Spotted Salamander), *Desmognathus quadramaculatus* (Black-bellied Salamander), *Eurycea l. longicauda* (Long-tailed Salamander), *Hemidactylium scutatum* (Four-toed Salamander), and *Plethodon cinereus* (Red-backed Salamander) within Wise County, Virginia.

TABLE 1: Comparison of all Amphibian Species Known to Occur in Wise County, Virginia.

Amphibian Species	Mitchell and	
	Reay(1999)	This Study
Anura		
Bufo a. americanus (Eastern American Toad)	X	X
B. fowleri (Fowler's Toad)	X	X
Hyla chrysoscelis (Cope's Gray Treefrog)	X	X
Pseudacris brachyphona (Mountain Chorus Frog)	X	
P. c. crucifer (Northern Spring Peeper)	X	X
Rana catesbeiana (American Bullfrog)	X	X
R. clamitans melanota (Northern Green Frog)	X	X
R. palustris (Pickerel Frog)	X	X
R. sylvatica (Wood Frog)	X	X
Caudata		
Ambystoma jeffersonianum (Jefferson Salamander)	X	
A. maculatum (Spotted Salamander)		X*
Aneides aeneus (Green Salamander)	X	
Desmognathus f. fuscus (Northern Dusky Salamander)	X	X
D. monticola (Seal Salamander)	X	X
D. ochrophaeus (Allegheny Mountain Dusky Salamander)	X	X
D. quadramaculatus (Black-bellied Salamander)		X*
D. welteri (Black Mountain Salamander)	X	
Eurycea cirrigera (Southern Two-lined Salamander)	X	X
E. l. longicauda (Long-tailed Salamander)		Х*
E. lucifuga (Cave Salamander)	X	X
Gyrinophilus p. porphyriticus (Northern Spring Salamander)	X	X
Hemidactylium scutatum (Four-toed Salamander)		X*
Notophthalmus v. viridescens (Red-spotted Newt)	X	X
Plethodon cinereus (Eastern Red-backed Salamander)		X*
P. glutinosus (Northern Slimy Salamander)	X	X
P. kentucki (Cumberland Plateau Salamander)	X	
P. richmondi (Southern Ravine Salamander)	X	X
Pseudotriton r. ruber (Northern Red Salamander)	X	X

<sup>\*</sup>Denotes new county record for Wise County, Virginia

#### DISCUSSION

Among the 23 amphibian species documented in this study, we interestingly report the first documented occurrences of A. maculatum, D. quadramaculatus, E. l. longicauda, H. scutatum, and P. cinereus in Wise County, Virginia (to the best of our knowledge). Though these species have not been reported previously, their occurrence within the county is not altogether surprising. The preferred habitats of mixed

deciduous forests (A. maculatum and P. cinereus), hardwood or coniferous forests (H. scutatum), and mountain stream (D. quadramaculatus and E. l. longicauda) are all found within Wise County, Virginia borders. In fact, Mitchell and Reay (1999) suspected that A. maculatum, D. quadramaculatus, and H. scutatum could be found once regions in southwest Virginia were properly surveyed. Our study directly answered this survey call.

Mitchell and Reay (1999) report the occurrence of four species that we did not find in our Wise County, Virginia survey. These species include: Pseudacris brachyphona (Mountain Chorus Frog), Aneides aeneus (Green Salamander), D. welteri (Black Mountain Salamander), and P kentucki (Cumberland Plateau Salamander). Altogether, the lack of discovery of these species in our study was not surprising. For example, P. brachyphona populations across its entire range are in apparent population decline due to deforestation and urbanization (Murdock 1994). Partially because of population decline concerns, the Commonwealth has strict regulations concerning P. brachyphona commercialization (Mitchell and Pauley 2005). Aneides aeneus is also heavily impacted by anthropogenic disturbances, partially because of its highly specific preferred habitat consisting of rocky outcrops (Pauley and Watson 2005a). While it is not considered a species of concern in the Commonwealth, adjacent states have A. aeneus listed as endangered or threatened. Plethodon kentucki prefer to occupy pristine, mature hardwood forests. Overexploitation of hardwood resources for commercial products has significantly impacted P. kentucki populations across its already small home-range (Pauley and Watson 2005b). Future studies and surveys could attempt to ascertain the population status of these anurans and caudates in Wise County, and southwest Virginia as a whole, since much of the region has herpetological unknowns.

While exceedingly rare in the primary literature, studies such as this survey are vital for amphibian population monitoring efforts. Thus, this study contributes to the herpetofaunal list of Wise County, Virginia, and to the Commonwealth itself, by potentially acting as a foundational baseline of data. However, much more work is needed in future years to monitor Wise County, Virginia amphibians. Population trends can only be established with several years of surveying. Even then, population declines may not obvious (Hairston and Wiley 1993). Nevertheless, these efforts are warranted because of the high degree of amphibian biodiversity and endemism within Wise County, Virginia and the surrounding Appalachian Mountains.

#### **ACKNOWLEDGMENTS**

Thanks are due to all the field volunteers for their assistance with various aspects of this project. We also wish to thank the landowners that gave us permission to transverse their private property. Funding for this project was awarded to SRAD by means of an undergraduate student Fellowship in the Natural Sciences at the University of Virginia's College at Wise. All observations were done in compliance with the Institutional Animal Care and Use Committee at the University of Virginia's College at Wise. The authors (SRAD and DLC) contributed equally to this project.

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### Modeling and Simulation on Signatures of Mars Minerals

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#### **ABSTRACT**

The objective of this study was to assess the feasibility of identiying minerals on Mars using remotely sensed data. In the process we also investigated the effect of noise of aerosol and dust particles on the spectra of Mars minerals. The remotely sensed data was obtained through modeling and simulation and compared to the lab spectroscopy of the specific minerals in order to make an accurate identification. A linear model was developed using MATLAB Random Number Generator to obtain a simulated image. Part of the information we needed for the linear model was pure pixel information of Mars which was obtained from Mars Spirit images. Random noise was added to the image in order to simulate a real world image. In addition to the random noise, a mathematical model was developed to represent the noise caused by aerosols and dust particles in Mars's atmosphere. The simulation was tested to ensure that it satisfied the appropriate model testing. Our results showed that our linear model was appropriate, and was accepted at a confidence interval of about 95%. The simulated image was then corrected from noise through iterations. The overall accuracy of the corrected image showed an improvement in classification by 25%. The signatures of the spectra of the two images were obtained and compared to the lab spectroscopy of specific minerals. The degradation of noise showed improvement in the spectral analysis of Mars data. The spectral analysis showed the presence of iron oxide, calcium oxide and magnesium oxide leading to the conclusion that the image simulation is reliable in mineral spectral identification.

#### **Key Words**

Remote sensing of planetary surface, spectroscopy, and mathematical modeling

#### INTRODUCTION

Remote Sensing aspect of space science and technology relies mainly on sensors on satellites and mounted in telescopes to monitor Earth, other planetary bodies and distant stars and galaxies. This research is important since extraterrestrial remote sensing may make the greatest contributions to useful knowledge of value to humankind's future. Remote sensing in time became an important means of analyzing the status of what was on the planet's surface: clues as to mineral content (Avery, et.

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al., 2001). Mars mineral identification is a growing area in scientific community. Researchers attempted to determine minerals using different approaches. However, mineral identification on Mars is underway through orbital visible-infrared remote sensing in concert with spectroscopic, chemical and magnetic measurements (J. Bishop, 2005). The objective of this research is to determine minerals in Mars in visible and the near-IR (near infrared) (0.35-1.4 micrometers) through modeling and simulation and remote sensing techniques as described in the Methods. The objective of this study was to identify minerals on Mars through developing a linear mixture model using remotely sensed data and compared it to the spectroscopy of the specific minerals in order to make an accurate identification . In the process we also investigated the effect of noise of aerosol and dust particles on the spectra of Mars minerals. The applications for this method are numerous, but the most significant would be to remotely determine the mineral make up of a planetary surface accurately.

#### **METHODS**

The data were extracted from the Spirit instrument MER-A (Mars Exploration Rover – A, January, 2004). In order to simulate an image composed of a mixture of minerals, end member spectra (EMS) and cover class proportions (CCP) were used.

Principle of Linear Mixture Model

In developing the simulated image, the linear mixture model approach was used. The linear mixture model includes mixtures of nine different classes for three sets of EMS representing minerals with different CCP. The requirement of the linear mixture model depends on the extraction of EMS and the CCP (Jian and Haigh 1997).

To extract the EMS from pure pixel values (X) in a homogenous part of the imagery, a certain number of training sets are predefined and each pixel is assigned to a training set that it resembles. The quality of training sets depends mostly on accuracy of the automated classifier (Lilisand and Kiefer, 1994).

If there are c types of ground cover and n spectral bands, it is always assumed that  $n \ge c$  to avoid the identifiability problem. A column vector  $f = [f_1, ...., f_c]^{\tau}$  is used to denote the proportions of areas within the pixels occupied by each of the c types of ground cover.

In correspondence with the Linear Mixture model, we can formulate the equation below:

$$x_i = \sum M_{ii} f_i + e_i \quad , \tag{1}$$

Where  $M_{ii}$  is independent of  $f_i$  and  $e_i$  represents noise.

We can rewrite equation (1) in matrix form as:

$$X = mf + e = \mu_1 f_1 + \mu_2 f_2 + ... + \mu_c f_c.$$
 (2)

to estimate  $\hat{f}$  which satisfies the constraints such that:

$$\sum_{j=1}^{c} \hat{f}_{j} = 1, \hat{f}_{j} > 0, \quad j = 1,...,c.$$
 (3)

In order to ensure that all the error is due to atmospheric noise, the least square method is used. The assumption is that the random noise is confined to E' and denoted as a column vector as  $E' = [E_I, ..., E2]$  Then equation (2) can be modified to be (Jian and Haigh 1997):

$$X' = \hat{M}F + E' . (4)$$

The error can be minimized by using:  $\|X' - MF'\|^2$ , (Jian, L., and Haigh, J. 1997). Several LS constraining methods were used to estimate the CCP which can be shown in the following.

#### 1. Normalized Least Squares Method

If the estimated  $\hat{F}_{LS}$  included a negative element of CCP, they will be set to zero, then the remaining elements will be scaled so that they all total one. For example, when c equals 4 classes, if a vector of the estimated CCP  $\hat{F}_{LS}$  is [ 0.4 -0.05 0.7 -0.06 ], then the negative proportions -0.05 and -0.06 will first be set to zero, so as to convert to [ 0.4 0 0.7 0 ]. Secondly, each proportion will be multiplied by 1 / {sum of elements (0.4+0+0.7+0)} to yield  $\hat{F}_{NLS}$  = [4/11 0 7/11 0]. Consequently, this is the closest point to  $\hat{F}_{LS}$  while satisfying the constraints of Equation (3), (Settle and Drake, 1994).

#### 2. Lagrangian Least Square Method

Settle and Drake (1994) proposed an algorithm to solve the constrained least squares problem. If the constraints of Equation (3) are satisfied, the new equation can be derived by the Lagrangian analysis, such that

$$\hat{F}_c = \alpha U j + (1 - U j) \hat{F}_{LS} , \qquad (5)$$

Where  $j = [1, ..., 1]^T$  in a  $c \times 1$  matrix where the elements are all 1,

 $J=jj^T$  is a c × c matrix I in a c × c identity matrix,  $U=(M^TM)^{-1}$  is a c × c matrix, and  $\alpha=(JUJ^T)^{-1}$  is a constant.

Eventually, the newly constrained least squares solution (  $\hat{F}_{L\!L\!S}$  ) can be decided such that:

$$\hat{F}_{LLS} = \hat{F}_c \qquad 0 \le j^T \hat{F}_c \le 1$$

$$= (I - \alpha UJ) \hat{F}_c \qquad 0 \ge j^T \hat{F}_c$$

$$= \alpha Uj + (I - \alpha UJ) \hat{F}_c \qquad I \le j^T \hat{F}_c$$
(6)

The solution using the Lagrangian method constraints only the sum to one condition, so the solution may include negative proportions for some elements. Therefore, after finding the solution by using Equation (6), the normalizing method that was discussed in the previous section can be applied for the negative elements.

#### 3. Weighted Least Squares Method

A constraint can be imposed as:

$$C = \|\hat{M}F - X\|^2 + \lambda^2 \|1 - jF\|^2 \tag{7}$$

With a very large weight factor,  $\lambda$ , so that, in a deviation from 1-j, F will cause a significant error to C. Consequently, the sum of one condition, j'F=1, is effectively imposed. Equation (7) can be written as the following matrix (Settle and Drake, 1994):

$$\mathbf{C} = \begin{bmatrix} 1 \\ \dots & 1 \\ \dots & \dots & 1 \\ \dots & \dots & \dots & 1 \end{bmatrix} \times \begin{bmatrix} \hat{M} \\ \vdots \\ f_c \end{bmatrix} - \begin{bmatrix} X_1 \\ \vdots \\ X_n \\ 1 \end{bmatrix} \end{bmatrix}^2 \tag{8}$$

$$C = \begin{bmatrix} \dots \hat{M} \\ \lambda_1 \dots \lambda_n \end{bmatrix} \times F - \begin{bmatrix} \lambda \\ X \end{bmatrix}^2$$
 (9)

Once the  $\,\hat{M}\,$  and  $\,\widetilde{X}\,$  minimizing equation (12) are found, then:

$$\hat{F}_{WLS} = \hat{M} + \tilde{X} \tag{10}$$

Where the subscript WLS represents weighted least squares,

$$\hat{M} = \left[\frac{\hat{M}}{\lambda_1 \dots \lambda_n}\right], \text{ and } \hat{X} = \left[\frac{X}{\lambda}\right]$$

#### Mathematical Atmospheric Model

The principle of the mathematical atmospheric model is that the light undergoes transformation and nonlinear change as it is scattered by aerosols while passing through the atmosphere. The set of eignvalues represents value of coefficient of the

scattering vector in space. The nonlinear change is proportional to the light intensity (Logan, 2006) as in the following:

$$I'' - \eta I = 0 \tag{11}$$

where  $\eta > 0$ .

The general solution for the intensity in equation (6) is;

$$I = A\cos\mu x + B\sin\mu x , \qquad (12)$$

where  $\eta=\mu^2$  , and its derivative is,

$$I' = -A\mu \sin \mu x - B\mu^2 \cos \mu x , \qquad (13)$$

$$I'' = -A\mu^2 \cos \mu x - B\mu^2 \sin \mu x , \qquad (14)$$

Substituting into equation (6), the following matrix is presented:

$$\begin{vmatrix} \cos \mu a - \cos \mu b & \sin \mu a - \sin \mu b \\ \sin \mu b - \sin \mu a & \cos \mu a - \cos \mu b \end{vmatrix} = 0$$
 (15)

We then solve for the eigenvalues:

$$\mu_n = \frac{2n\pi}{b-a} \tag{16}$$

$$\eta_n = \left(\frac{2n\pi}{b-a}\right)^2 \tag{17}$$

The eigenvalue is assumed to be equal to the optical depth in an atmospheric layer as in the following:

$$\eta_n = \frac{K_{sca} \exp(-Z/H) * A * Z}{\cos \theta}$$
 (18)

where  $K_{\text{sca}}$  is the scattering coefficient. Z is the altitude in kilometers, and  $\theta$ , represents the zenith angle (Bohren and Huffman, 1983). This equation can be used for computation of the atmospheric noise that is intercepted by the telescope or the sensor. The atmospheric noise is represented by the following:

$$\delta = \frac{(1 + Area)K_{sca}A * B * Z * T^{pixel}}{(1 + area)K_{sca}A * B * Z + 1}$$
(19)

The atmospheric noise will be added to the linear mixture model equation as in the following equation:

$$X' = \hat{M} F + E' + \delta \tag{20}$$

As we mentioned previously this atmospheric noise will be degraded using iteration, trial and error iterations. The accuracy of correction can be measured by the overall accuracy of classification. The corrected image signatures will be compared to the lab spectra of minerals.

#### Simulation of Linear mixture model

The EMS was obtained by selecting pure pixel values from a perfectly homogenous area of Mars Spirit images, which represent different minerals. Perfectly homogenous areas are designated by similar signatures/spectral values and/or spectrally separable classes as shown by EMS data sets in Table 1. The extracted data set 1 (EMS set 1) consisted entirely of spectrally separable classes with distict signature values (threshold • 10 signature values) as shown by band 3 (class1, class 2, and class 3). The EMS data set 2 (EMS set 2) is made up of a mixture of spectrally separable (band 1-class3, band 2-class 3, and band 3-class 3) and similar classes (threshold • 6 signature values). The data set 3 (EMS set 3) consisted entirely of spectrally similar classes and do not show any distinct values. We obtained samples of the necessary training sets within the simulated image by using the training set signature editor in ENVI 4.4 (commercial software) where a reference cursor on the screen was used to manually delineate training area polygons in the displayed image. The pixel values within the polygons were used in the software to develop a statistical description file for each training area. The next step is estimate the CCP using the different constraining methods as described previously. Furthermore, to impose the critical constraints, which are the "sum to one" and "make all CCP positive," several methods were used, such as the Normalization, Lagrangian, and Quadratic constraining methods and the weighted constraining method (Settle and Drake, 1994). These methods were tested to determine the best constraining method for this experiment. After deciding upon CCP estimation and constraining methods, evaluations of estimating EMS and its effects on the corresponding CCP estimation were presented in the section above.

The Quadratic Programming method was tested to be the best constraining method as described in linear mixture model. Using the EMS data sets, the pixel values were computed based on Equation (2) using the MATLAB Random Number Generator. The mathematical model was used to derive the atmospheric error. The atmospheric noise errors were added to the EMS to simulate realistic data sets. As mentioned previously, a minimization of the random error in pixel value was implemented (section 2.1). An ASCII file with pixel values was produced and imported in ERDAS IMAGINE as an image file. The image is then corrected for atmospheric noise through trial and error iterations to reduce the atmospheric error and produce enhancement to the classification accuracy. The Maximum Likelihood automated classifier in ENVI (commercial software) was used. At each iteration step, the atmospheric noise was subtracted using initial values of solar zenith angles and scattering coefficient. The iterations were terminated when the overall classification accuracy reached an optimum value. The signature graphs of the corrected image are compared with lab spectra of minerals (Dalton et. al, 2005). The lab spectra of minerals are considered a "fingerprint" (Clark, 1983). If the graph behavior of corrected image signature values is matching the lab spectra of minerals, then we can conclude that the mineral is identified.

#### RESULTS

Three different EMS data sets or classes with nine subsets were generated in Table 1. After adding the random noise, the CCP was normalized again to make all CCP positive and equal one. The CCP was estimated using the least squares and end member spectra method (LS EMS) with different constraining methods. To avoid an unexpected random effect, we repeated the calculation several times for each noise level. The Roat Mean Square Error (RMSE) of the different combinations of CCP constraining methods by changing the noise level while using the same LS-EMS is shown in Figure 1. The different combinations of CCP constraining methods are L-LS-CCP (Lagrangian-Least Square-Cover Class Proportions), W-LS-CCP (Weighted-Least Squares-Cover Class Proportions) and Q-CCP (Quadratic programming constraining method-cover class proportions).

The different sample sizes may affect the overall results of these experiments. So, the same experiment was performed with changing the sample sizes. The RMSE of different combinations of the CCP estimation and constraining methods by changing the sample sizes while using the same LS-EMS is shown in Figure 2.

The results show that the quadratic programming method proved to be the best constraining method for CCP estimation. This is shown in Figures 1 and 2, indicating that this method performed much better because of a lower (Roat Main Square Error) RMSE, which does not change with sample size. The result was reasonable, because of adding a normally distributed error and testing the sample groups that created the data set.

The simulated image including the atmospheric effect was tested using statistical testing for appropriate model and significant regression model using SAS/STAT software. We presented some selected samples of results which show the regression is significant at 90% confidence interval. Since  $F_{k\cdot 2,n\cdot k}$  is larger than 14.25 (Milton and Arnold,1986).  $H_0$  is accepted at p < 0.025 at 97.5% confidence. Therefore it can be concluded that the model is appropriate.

The simulated image for minerals with atmospheric noise/effects is shown in Figure 3. The corrected simulated image from atmospheric effects is shown in Figure 4. The correction accuracy is presented by the overall accuracies of classification at the final iteration which are shown in Tables 2, 3, 4, 5, 6, and 7. The overall accuracy of classified pixels for the image with atmospheric effect (Figure 3) is 71.42% and for the corrected simulated image (Figure 4) is 97.56%. The overall accuracy shows improvement in classification which ranges between 22% -25%. The wavelength was plotted versus the spectral signature (spectral radiance) as in Figures 5, 6, and 7.

#### DISCUSSION AND CONCLUSION

Since the statistical regression test was conducted at 90 % confidence interval for the linear mixture model, we conclude that the regression model is significant. We conclude that the model is appropriate at 97.5 confidence interval. The results of classifications were presented by the error matrices for three minerals as in Tables 2, 3, 4, 5, 6, and 7. In each table, the last row includes the column total represents the truth data. The diagonal and no diagonal elements represeant the classified data The spectral values along the diagonal are higher than the off diagonal ones which indicated

higher accuracy. The overall accuracy showed improvement by 22%-25%. This indicates that the accuracy of correction is significant. We can conclude that the correction using the atmospheric model produced significant classification accuracy. Figures 5, 6, and 7 represent the wavelength versus signatures (spectral radiances) of the simulated image for three minerals that were compared with the experimental spectra of different minerals. The signatures matched the experimental lab spectra for iron oxide, magnesium oxide and calcium oxide. Thus, the spectral analysis showed the presence of iron oxide, calcium oxide and magnesium oxide leading to the conclusion that the image simulation is reliable in mineral spectral identification. The applications for this method are numerous, but the most significant would be to remotely determine the mineral make up of a planetary surface accurately.

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This work was under the supervision of Dr. W. Elmahboub in the Mathematics Department at Hampton University. Mr. Yankey assisted in developing the linear mixture model using MATLAB and ENVI 4.4. Ms Kerwin assisted in writing the first draft of this paper.

TABLE 1. EMS set1, 2, and 3

EMS	Sets	Band 1	Band 2	Band 3	Label
EMS set 1	class 1	54	50	67	subset 1
	class 2	58	57	71	subset 2
	class 3	59	55	101	subset 3
EMS set 2	class 1	142	146	150	subset 4
	class 2	148	150	154	subset 5
	class 3	131	131	144	subset 6
EMS set 3	class 1	241	242	246	subset 7
	class 2	245	243	249	subset 8
	class 3	257	250	252	subset 9

TABLE 2 Corrected image of mineral 1. Higher diagonal elements than non diagonal pertain to higher accuracy being achieved with correction compared to Table 3.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	12	0	0	12
Min. 2	1	19	0	20
Min. 3	0	0	9	9
Column Total (truth data)	13	19	9	41

TABLE 3 Classification of the noisy image of mineral 1. Less diagonal elements and more non diganoal elements compared to Table 2 which pertain to less accuracy.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	11	8	1	20
Min. 2	2	9	0	11
Min. 3	0	2	8	10
Column Total (truth data)	13	19	10	41

TABLE 4 Classification of corrected image of mineral 2. Higher diagonal elements than non diagonal pertain to higher accuracy being achieved with correction compared to Table 5.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	12	0	0	12
Min. 2	0	16	0	16
Min. 3	0	0	11	11
Column Total (truth data)	12	16	11	39

TABLE 5 Classification of the noisy image of mineral 2. Less diagonal elements and more non diganoal elements compared to Table 4 which pertain to less accuracy.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	7	5	0	12
Min. 2	5	11	0	16
Min. 3	0	0	11	11
Column Total (truth Data)	12	16	11	39

TABLE 6 Classification of the noisy image of mineral 3. Higher diagonal elements than non diagonal pertain to higher accuracy being achieved with correction compared to Table 7.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	7	5	0	12
Min. 2	1	13	0	14
Min. 3	0	0	7	7
Column Total (truth data)	8	18	7	33

TABLE 7 Classification of corrected image of mineral 3. Less diagonal elements and more non diganoal elements compared to Table 6 which pertain to less accuracy.

Classified Data	Min. 1	Min. 2	Min. 3	Row Total
Min. 1	8	0	0	8
Min. 2	0	18	0	18
Min. 3	0	0	7	7
Column Total (truth data)	8	18	7	33

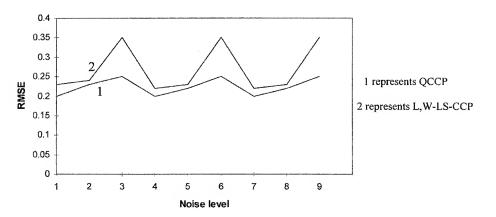


FIGURE 1. RMSE of CCP estimation and constraining methods

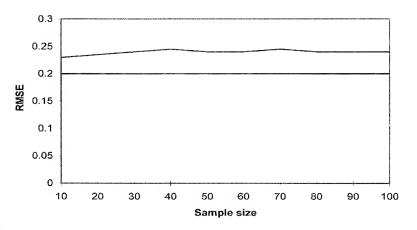


FIGURE 2. RMSE of CCP estimation and constraining methods by changing sample sizes

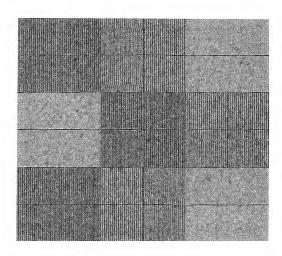


FIGURE 3. The simulated image with atmospheric noise.

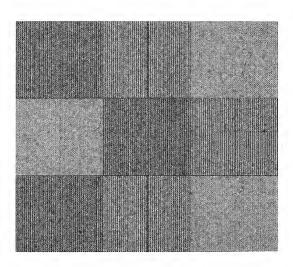


FIGURE 4. Corrected simulated image.

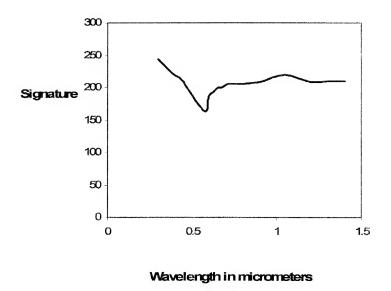


FIGURE 5. Wavelength in micrometer versus signature (in spectral radiance) shows the presence of calcium oxide when compared to lab spectra of USGS.

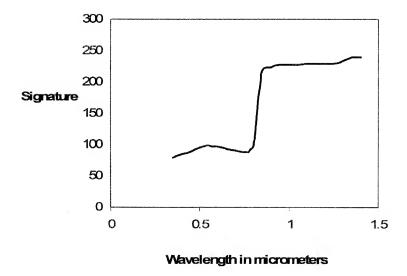


FIGURE 6 Wavelength in micrometers versus signature (in spectral radiance) shows the presence of iron oxide when compared to lab spectra of USGS.

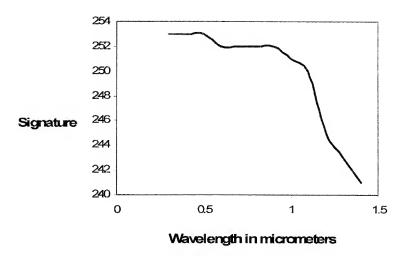
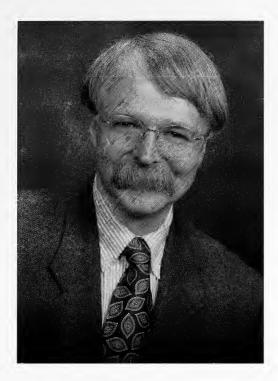


FIGURE 7. Wavelength in micrometer versus signature (in spectral radiance) shows the presence of magnesium oxide when compared to lab spectra (USGS).



#### **THOMAS ORR SITZ (1944-2010)**

Past Academy President (1995-1996), Dr. Thomas Orr Sitz, of Blacksburg, Virginia died Tuesday, September 28, 2010 at Montgomery Regional Hospital. Born in Newport, Rhode Island on December 9, 1944 to the late John Harry and Margaret McCullough Sitz. He received a B.S. in Biology from Virginia Tech in 1967 and a Ph.D. in Biochemistry in 1971. Dr. Sitz received a prestigious post-doctoral appointment at Baylor College of Medicine in Houston, Texas under Dr. Harris Busch and was later appointed to a faculty position from 1971-75. He then was a member of the Chemistry Department at Old Dominion University in Norfolk, Virginia where he continued his research from 1975-82. Dr. Sitz recently retired from Virginia Tech and was granted Professor Emeritus. He was Associate Professor of Biochemistry and Anaerobic Microbiology in the College of Agriculture and Life Science, and Director of Premedical Studies. He was the recipient of the Alumni Award for Excellence in Undergraduate Advising in 1982.

He is survived by his wife, Bonnie Crabtree Sitz of Blacksburg; daughter and son-in-law, Molly Sitz Moore and husband Rear Admiral Scott P. Moore of Virginia Beach; sisters, Lucy Aginiga of Chula Vista, California, Margaret Cullifer of Hatteras, North Carolina; brother, John Sitz of Ocracoke, North Carolina; grandchildren, Rachel Laughlin Moore, Sarah Buckley Moore, Thomas Dewey Moore, IV.

Dr. Sitz was a strong supporter and treasured asset to the Academy. He will be greatly missed.

#### Establishment of Thomas O. Sitz Memorial Student Award

When Thomas O. Sitz died in September, after a long battle with cancer, the Virginia Academy of Science and Virginia Tech lost a wonderful colleague andan inspirational friend. He was Associate Professor of Biochemistry and Anaerobic Microbiology and Director of Premedical Studies at Virginia Tech. He served as President of VAS in 1995-1996, was a Fellow of the Academy, and provided leadership and brought his students to the Chemistry Section for many years. The Council of the Academy proposes to honor Tom by establishing a VAS Student Award in memory of his scientific work, his generous teaching, and his joyous spirit. We hope that Tom's colleagues and friends will feel this is a fitting recognition of what his life and example have meant to all of us. Just as Tom focused his energies on developing the next generation of scientists and science education, so, too, does this award seek to serve that noble purpose. To assist the Academy in funding the Sitz Award, we request that you send your contributions to:

Dr. Arthur Conway, Executive Officer Virginia Academy of Science Science Museum of Virginia 2500 West Broad Street Richmond, Virginia 23220

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# Functional Feeding Groups, Species Richness and Spatial Distributions of Fishes in Rocky and Sandy Beach Habitats of St. John, U.S. Virgin Islands

Eugene G. Maurakis<sup>1,2</sup>, George E. Maurakis<sup>3</sup>, and Demetri E. Maurakis<sup>4</sup>

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#### **ABSTRACT**

Objectives were to identify and compare fish species richness, functional feeding group richness and diversity, and delineate distributions of fishes at rocky and sandy beach habitats at St. John, U.S. Virgin Islands. Visual observations using snorkel and mask were made at 3-m intervals seaward from shore during daylight hours. A total of 69 taxa (67 species) representing 33 families of fishes were observed. Total (53) and average fish species richness (32.7) at rocky beach habitats were greater than those (total=43; average=24.3) at sandy beach habitats. Twelve functional feeding groups were identified (diurnal planktivores, excavators/eroders, macroalgae browsers, macrocarnivores, mobile benthic invertivores, general omnivores, strict piscivores, sand invertivores, scrapers, coral/colonial sessile insectivores, territorial algae/detritus, and turf grazers). Total numbers of functional feeding groups (range=10-12) and species (range=29-46) per functional feeding groups at distances greater than 1 m from shore at rocky beach habitats were consistently higher than those (functional feeding group range=8-10; species per functional feeding group=19-30) at sandy beach habitats. Information on the number and composition of functional feeding groups in rocky and sandy beach habitats from this study can serve as a baseline for future investigations as changes in Caribbean habitats continue to occur.

Keywords: fish species richness, fish functional feeding group, Caribbean beach habitats

#### INTRODUCTION

Substrate complexity such as that offered by coral reefs is significant in providing diverse habitats that harbor a variety of fishes, particularly reef fishes (Christensen et al., 2003; Claro et al., 2001; Friedlander and Parrish, 1998; Gratwiche and Speight,

2005; Mac et al., 1998; Monaco et al., 2003; 2007; Nero and Sealey, 2005; Ohman and Rajasuriya, 1998). Other habitat areas, such as Caribbean mangroves and seagrass beds, are also important habitats as they serve as nursery and feeding areas for a variety of juvenile reef fishes (Faunce and Serafy, 2006; Nagelkerken and van der Velde, 2002). Focus on the decline of coral reefs and mangroves and the various ecological functions they provide to reef fishes has overshadowed habitats (i.e., sandy and rocky nearshore habitats) considered to be lesser important in understanding the health and dynamics of tropical ecosystems and their ichthyofaunas (Nero and Sealey, 2005). Granted, fish communities in sandy and rocky beaches have relatively depauperate ichthyofaunas compared to those of reef systems. Sandy and rocky nearshore habitats, however, harbor some of the same fish species common to reefs, mangroves, and seagrass beds (Valdez-Munoz and Mochek, 2001; Ortiz and Lalana, 2008). Except for some observational data on selected species in nearshore sandy and rocky Caribbean habitats provided by Valdez-Munoz and Mochek (2001), there is a paucity of published information on the fishes in sandy and rocky beach habitats in the Caribbean. Knowledge of species richness and distributions of fishes in nearshore sandy and rocky-shore habitats can also serve as baseline data for future comparisons as changes occur in reef, mangroves, and seagrass habitats related to chronic anthropogenic impacts (e.g. overfishing, habitat degradation) and climate change. For example, upwards of 90 % of reefs of the U.S. Virgin Islands (USVI) experienced bleaching in 2005 when sea surface temperatures were higher than the previous 14 years (Rothenberger et al., 2008). Of particular importance may be the number and composition of functional feeding groups in these habitats, where the loss of one or two functional feeding groups represented by one or few species could be critical to the functioning of the ecosystem (Halpern and Floeter, 2008).

Objectives of this study were to identify and compare fish species richness, functional feeding group richness and diversity, and delineate distributions of fishes at sand- and rock-shoreline beach habitats at St. John, U.S. Virgin Islands in the Caribbean Sea.

#### MATERIALS AND METHODS

Fish species richness and spatial distributions were surveyed by visual census using snorkel and mask at each of 1, 3, 6, 9, 12, 15, and 20 m from shore at each of 30 transects (14 sandy- and 16 rocky-shoreline habitats) at Little Lameshur (18.32026 N, -64.72551 W), Great Lameshur (18.31822 N, -64.72427 W), and Francis Beach (18.36537 N, -64.74365 W), St. John, USVI, during daylight hours (i.e., 0800-1800) from 12-18 July 2007 and 8-18 July 2008. Transects were established randomly at each rocky or sandy beach habitat, and none were re-sampled during the second year. Low light conditions and poor visual acuity at greater depths precluded the recording of species beyond 30 m from shore. The vertical observational zone was from the bottom substrate to the surface, including boulder ledges and crevices. No substrate material was overturned or dislodged. Fishes within an estimated 3-m horizontal circumference of the observer were identified by visual observation. identifications were verified by examining digital photographs made underwater with an Olympus Stylus 770 SW or Olympus 850 SW camera at each distance per transect. Water depth (m) was measured with a weighted cord marked in 1-m increments. Relative percents of habitat composition (i.e., sand, gravel, cobble, boulder, seagrass,

and coral) were estimated by observation and recorded at each distance from shore. These percents were transformed to their arcsin equivalents prior to statistical analysis. Fish census data are available upon request.

Assignment of species to functional feeding groups (diurnal planktivores, excavators/eroders, macroalgae browsers, macrocarnivores, mobile benthic invertivores, general omnivores, strict piscivores, sand invertivores, scrapers, coral/colonial sessile insectivores, territorial algae/detritus, and turf grazing) follows the designations in Halpern and Floeter (2008). Species richness, and functional feeding group richness, Shannon-Weiner diversity and evenness were compared among (general linear model followed by Duncan's Multiple Range Test at p=0.05, SAS, 2009) and between (t-test at p=0.05, SAS, 2009) rocky and sandy shore habitats.

#### RESULTS

Average water depths (1.6-4.6 m) at each distance from shore (3, 6, 9, 12, 15, and 20 m) at rocky transects were significantly greater than those (0.94-2.5 m) at sandy transects (Table 1). Percent occurrences of coral, boulder and seagrasses at rocky habitats were significantly higher than those from 3-20 m from shore at sandy habitats with three exceptions (Table 1). Percent occurrence of coral at 15 and 20 m and that of seagrasses at 20 m from shore did not vary significantly between rocky and sandy habitats (Table 1). Conversely, percent occurrences of cobble, gravel, and sand at rocky habitats were significantly lower than those at sandy habitats from 3-20 m from shore with two exceptions. Occurrence of sand and gravel at 20 m from shore did not vary significantly between rocky and sandy habitats (Table 1).

A total of 69 taxa (67 species + 2 families) representing 33 families of fishes were observed (Table 2) in the 30 sandy and rocky transects at Little Lameshur, Francis Beach, and Great Lameshur, St. John, USVI. The most speciose families were Scaridae (8), Haemulidae (7), Pomocentridae (6), Labridae (4), and Lutjanidae (4). Seventeen families were each represented by one species. Total fish species richness at rocky habitats was 53; that at sandy habitats was 43. Average number of species (32.7) at rocky habitats was significantly greater than that (24.3) at sandy habitats (t=6.18; p=0.0016). Species richness (avg. range=11-20) at combined rocky habitats did not vary significantly at distances 6-20 m from shore (Table 3). In contrast, at combined sandy habitats, species richness (avg. range=1-5.9) did not vary significantly at distances 1-20 m from shore (Table 4). Species richness (avg. range 3-20) at each distance from shore at rocky habitats was significantly greater than those (avg. range=1-5.9) at sandy habitats (Table 5).

Numbers of functional feeding groups encountered at rocky habitats were consistently higher than those at sandy habitats (Tables 6-7). On average, rocky habitats from 3-20 m from shore had two more functional feeding groups (avg.=11.3) than sandy habitats (avg.=9.3; Tables 6-7). Two species (*Archosargus rhomboidalis* and *Sparisoma radians*) of the macroalgae browser functional feeding group occurred frequently at rocky habitats. No macroalgae browsers were observed at sandy habitats. The most species functional feeding groups were mobile benthic invertivores (11 species at 9 m in rocky habitats), scrapers and piscivores (each 9 species at 6 m in rocky habitats), and macrocarnivores (6 species at 6 m in sandy habitats)(Table 2). Total numbers of functional feeding groups (range=10-12) and species (range=29-46) per functional feeding groups at distances greater than 1 m from shore at rocky habitats

were consistently higher than those (functional feeding group range=8-10; species per functional feeding group=19-30) at sandy habitats (Tables 1, 6-7). At the 1-m distance, functional feeding group richness at rocky habitats was five; that of the 1-m sandy habitat was one.

Except at 20 m from shore, functional feeding group richness (avg. range=6.67-10) and Shannon diversity (avg. range=1.84-2.05) from 3-15 m from shore at rocky habitats were significantly higher than functional feeding group richness (avg. range=5.33-8.33) and Shannon diversity (avg. range=1.43-2.02) at sandy habitats (Table 8). In contrast, functional feeding group richness (avg.=7.3) and diversity (avg.=1.86) at 20 m from shore at sandy habitats were significantly greater than functional feeding group richness (avg.=6.67) and diversity (avg.=1.53) 20 m from shore at rocky habitats (Table 8). Functional feeding group evenness indices (avg. range=0.8896-0.9171) at distances 6-20 m from shore at rocky habitats were significantly lower than those (avg. range=0.9226-0.9551) at sandy habitats (Table 8), indicating greater variability in numbers of species comprising the functional feeding groups in rocky habitats. For example, at 6 m from shore at rocky habitats, mobile benthic invertivores and scrapers totaled 10 and 9, respectively, whereas other functional feeding groups were composed of 1-5 species. At 3 m from shore, the average functional feeding group evenness index (0.9764) at rocky habitats was significantly greater than that (0.9539) at sandy habitats (Table 8).

#### DISCUSSION

Comparison of rocky and sandy beach habitats

The more complex habitats of the intermingled boulder, rock, and coral substrates at rocky habitats exhibited higher species richness and functional feeding group richness than did less complex sandy habitats. Rocky shore habitats, where fish species richness was correlated with increasing water depth and the presence of coral, boulders, cobble, and gravel, harbored more fish species (avg.=32.7) than did sandy habitats (avg.=24.3). Even at greater distances from shore (i.e., 15 and 20 m) at sandy habitats where the percentages of coral (6.0-6.2) and seagrass (26.0 at 20 m) were comparable to those (coral=5.5-7.4; seagrass=23.5 at 20 m) at the same depths at rocky beaches, species richness (avg. range=3.73-5.89) still was significantly lower than those (avg. range=11.0-12.8) at rocky habitats. That more complex habitats support greater fish species richness has been documented repeatedly in the literature (Christensen et al., 2003; Claro et al., 2001; Friedlander and Parrish, 1998; Gratwiche and Speight, 2005; Monaco et al., 2003; 2007; Nero and Sealey, 2005; Ohman and Rajasuriya, 1998; Valdez-Munoz and Mochek, 2001). Results from the present study are comparable to those of Gratwicke and Speight (2005) who studied the relationship between fish species richness and habitat complexity in a series of shallow tropical marine habitats in the British Virgin Islands. The lower species richness (range = 1-30) at sandy beach habitats is comparable to the findings of Valdes-Munoz and Mochek (2001) who reported low fish species diversity in non-estuarine sandy beach areas of Cuba where species richness was 25. Although vertical relief of substrates (e.g. boulder rock substrates) was not measured in the present study, average depth, distance from shore and percentage of rock were correlated with high species richness at rocky habitats. These results are not unlike those of Brokovich et al. (2006), who indicated that reef fish assemblages in the northern tip of the Red Sea varied between habitats, and that fish community structure was best explained by average depth, distance from shore, vertical relief, percent cover by rock, and cover complexity index.

Species richness and both the number and composition of functional feeding groups in rocky and sandy habitats may have applications in future studies as changes in Caribbean habitats continue to occur. For example, Halpern and Floeter (2008) point out that knowledge of the functional feeding groups provide insight into the assembly, structure and dynamics of ecological communities, and that the addition or loss of a few species can have significant to minimal impacts on ecosystem function. On average, rocky habitats from 3-20 m from shore had two more functional feeding groups (avg.=11.3) than did sand habitats (avg.=9.3). However, the numbers of species comprising the functional feeding groups at rocky habitats averaged 13.3 species (range 5-46) more than those at sandy habitats (range 1-30). In both rocky and sandy habitats, many functional feeding groups (i.e., turf grazer, excavator eroder, macroalgae browser, and territorial algae detritivore) were represented by only one or two species, with most single species functional feeding groups occurring in sandy habitats (Table 2).

Spatial and behavioral comparisons of fishes

Spatial and behavioral descriptions, and occurrences of diurnal inshore pelagic fishes, epibentic pomacentrids, suprabenthic resident reef fishes, and territorial benthic fishes of the Cuban shelf provided by Valdes-Munoz and Mochek (2001) present the single most detailed source for comparisons with fishes in rocky and sandy beach habitats in our study.

Diurnal inshore pelagic fishes

Valdes-Munoz and Mochek (2001) reported the diurnal, transient belonid, carangid, and sphyraenid species common in the inshore upper water column at study sites in Cuba. We encountered these same transient taxa at our inshore rocky and sandy habitats, but also observed atherinid, engraulid, and clupeid (e.g. *Harengula humerali*) schools in the upper water column at these habitats as well.

Diurnal Epibenthic pomacentrids

The epibentic pomacentrid, Abudefduf saxatilis (sergeant major) was reported by Valdes-Munoz and Mochek (2001) to be common on irregular bottom types and frequently formed large schools 95 % of the time they were observed. In contrast, we encountered individual A. saxatilis and never observed schools of A. saxatilis over nearshore rocky or sandy substrates. Valdes-Munoz and Mochek (2001) observed the epibenthic pomacentrid (blue chromis), Chromis cyanea (abundant in the Caribbean), forming large schools. We never observed a single C. cyanea at any of our rocky or sandy habitats, suggesting that these habitat substrates are not favorable to this species. Diurnal suprabenthic fishes

Suprabenthic fishes (i.e., scarids, acanthurids, labrids, and chaetodontids), diurnally foraging above the bottom, were the resident fishes living over reefs serving as the primary representatives of the reef fish community studied by Valdes-Munoz and Mochek (2001). They reported scarids occurring in small groups or alone, constantly moving over great distances during the day while foraging on coral; in sea grass beds; however, some scarid species (e.g. *Sparisoma radians*) showed some behavioral elements of nomadic fishes, a high degree of motor activity, and the formation of large schools. We never observed large schools of any of the scarids (i.e., *Scarus iserti*, *Scarus taeniopteryx*, *Sparisoma viride*, *Sparisoma aurofrenatum*, *Sparisoma* 

frondosum, Sparisoma radians, and Sparisoma rubripinne) in our study. We observed these species foraging on coral singly or within close proximity of other scarids. Three scarid species (S. aurofrenatum, S. frondosum, and S. radians) occurred at rocky habitats but were never observed at sandy habitats in our study.

Our observations of the acanthurids (*Acanthurus bahianus*, *Acanthurus chirurgus*, and *Acanthurus coeruleus*), spending most of their time on the bottom while grazing during the day, are consistent with the behaviors of the species reported by Valdes-Munoz and Mochek (2001). Whereas we observed some intraspecific aggression between individuals in these species, we never observed the formation of schools reported by Valdes-Munoz and Mochek (2001) probably because of the low density of individuals in the rocky and sandy habitats we studied.

Labrids, reported by Valdes-Munoz and Mochek (2001) to be one of the most common families foraging during the daytime, were common in both rock and sand beach habitats in our investigation. Five labrid species, *Halichoeres bivittatus*, *Halichoeres maculipinnia*, *Halichoeres radiatus* (juveniles only), *Lachnolaimus maximus*, and *Thalassoma bifaciatum*, were constantly on the move in search of food. In particular, our observations of *H. bivittatus* and *T. bifaciatum* are consistent with the movements and behaviors described by them, where the latter species was reported to be in a fast and constant motion for 99 % of their time while foraging over reefs on the Cuban shelf. We cannot, however, confirm their observations of group formation in *T. bifaciatum*.

Our observations of diurnal feeding behaviors near the bottom by *Chaetodon striatus*, *Chaetodon capistratus*, and *Chaetodon ocellatus* are consistent with those described by Valdes-Munoz and Mochek (2001). We did not observe any of these chaetodontids protecting territories, consistent with the report by Valdes-Munoz and Mochek (2001).

Diurnal territorial benthic fishes

Juvenile to adult *Stegastes leucostictus* (beaugregory) were observed to protect their territories against conspecific individuals during the daytime. These observations are consistent with descriptions of aggression of this territorial benthic species reported by Valdes-Munoz and Mochek (2001). *Stegastes diencaeus* (longfin damselfish) were commonly observed defending the confines of basket sponges in both rocky and sandy beach habitats.

Diurnal observations of nocturnal suprabenthic fishes

This group of primarily nocturnal species is composed of lutjanids, haemulids, and holocentrids. Valdes-Munoz and Mochek (2001) indicated that grunt and snapper aggregations are the largest among the species associated with the bottom, and are most active at night when they move off reefs and forage in neighboring areas. In our daytime study, small to large (>300 individuals) motionless or slow moving schools of juvenile to adult *Haemulon flavolineatum* (French grunt) and juvenile *Haemulon sciurus* (bluestripe grunt) occurred at both rocky and sandy beach habitats, usually in areas of cover such as overhanging boulders, submerged trunks of fallen trees or rock ledges. Adult *H. sciurus*, *Haemulon melanurum* (cottonwick) and *Haemulon parra* (sailor's choice) were observed usually as single individuals, not in schools. Only once did we observe two adult *H. parra* under a boulder. Juvenile *Lutjanus synagris* (lane snapper) and juvenile *Ocyurus chrysurus* (yellowtail snapper) were observed in schools hovering over the bottom. Adult *L. synagris* and *O. chrysurus*, as well as adult

Lutjanus analis (mutton snapper) and Lutjanus apodus (schoolmaster) were observed individually, not in schools. These observations of juvenile and adult grunts and snappers in our study areas are consistent with the findings of Valdes-Munoz and Mochek (2001) who reported the activities of these species in Cuban reef systems.

The nocturnal holocentrids, *Holocentrus rufus* (longspine squirrelfish) and *Myripristis jacobus* (blackbar soldierfish) were common to both rocky and sandy habitats. They were seen underneath overhanging rock ledges or under boulders where they remained motionless. They were frequently accompanied in these protected areas by juvenile and adult *H. flavolineatum* and *H. sciurus*.

Notes on other species at beach habitats

Individual *Synodus saurus* (bluestripe lizardfish) frequented sandy nearshore areas where they buried themselves tail first into the sand. Remaining motionless with only their eyes exposed, they ambushed small fishes that were within striking range, which was about equal to their total body length. Also common in sandy substrates were two bothids, *Bothus lunatus* and *Bothus ocellatus*. These flatfishes buried themselves in the sand at nearshore sandy habitats too, where they laid motionless with only their eyes exposed above the sand to ambush passing fishes. Individual or groups of up to three *Pseudopeneus maculates* (spotted goatfish) were common foragers in sand areas of both habitats studied. Two carangids, *Caranyx ruber* (bar jack) and *Trachinotus goodei* (palometa) were also common pelagic species at both beach habitats.

Although the blenniid (*Scartella cristata*) and the gobiid (*Bathygobius soporator*) were recorded from both rocky and sandy habitats less than three times each, their occurrences were probably underrepresented because of their cryptic behaviors and small sizes. Similarly, low frequencies of blenniid and gobiid species were also reported by Lindeman and Snyder (1999) in a study of nearshore hard bottom fishes of southern Florida.

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TABLE 1. Comparison of water depth (m), and percent cover of substrate type between rocky and sandy beach habitats at combined Little Lameshur, Francis and Great Lameshur beaches, St. John, USVI in July 2007 and July 2008.

Parameter	Distance from shore (m)	Rocky	Sandy	F	p > F
	1				
Depth		0.20	0.17	0.14	0.7247
Coral		0.0	0.0	-	-
Boulder		81.7	0.0	2.21	0.007
Cobble		3.3	0.0	1.0	0.3739
Gravel		0.0	10.0	3.0	0.1583
Sand		15.0	90.0	135.0	0.0003
Seagrass		0.0	0.0	-	-
0	3				
Depth		1.56	0.94	6.40	< 0.0001
Coral		1.10	0.00	3.91	0.0514
Boulder		49.60	0.00	392.47	< 0.0001
Cobble		10.60	32.20	21.99	< 0.0001
Gravel		4.40	30.00	19.58	< 0.0001
Sand		17.80	33.80	8.00	0.0059
Seagrass		12.60	0.00	60.32	< 0.0001
	6				
Depth		1.91	1.10	23.97	< 0.0001
Coral		3.42	0.00	34.00	< 0.0001
Boulder		48.30	0.00	3105.74	< 0.0001
Cobble		11.40	40.70	69.47	< 0.0001
Gravel		4.02	10.68	8.29	0.0045
Sand		13.84	43.18	76.09	< 0.0001
Seagrass		12.68	5.15	17.88	< 0.0001
	9				
Depth		2.84	1.22	103.28	< 0.0001
Coral		2.92	0.00	33.21	< 0.0001
Boulder		43.00	1.50	4957.44	< 0.0001
Cobble		5.17	22.29	40.24	< 0.0001
Gravel		2.50	6.88	28.39	<0.0001

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TABLE 1 continued

Parameter	Distance from shore (m)	Rocky	Sandy	F	p > F
Sand		17.17	64.00	282.35	< 0.0001
Seagrass		24.58	5.57	64.46	< 0.0001
	12				
Depth		2.76	1.29	89.87	< 0.0001
Coral		7.46	0.00	104.02	< 0.0001
Boulder		48.31	0.00	5870.34	< 0.0001
Cobble		6.76	34.17	33.87	< 0.0001
Gravel		3.38	5.42	3.99	0.0484
Sand		12.54	58.47	115.23	< 0.0001
Seagrass		7.18	0.97	10.32	0.0017
	15				
Depth		4.10	2.00	175.68	< 0.0001
Coral		5.47	6.23	0.20	0.6532
Boulder		38.52	6.23	362.87	< 0.0001
Cobble		4.06	31.98	44.00	< 0.0001
Gravel		1.02	3.02	18.43	< 0.0001
Sand		22.42	40.28	14.23	0.0003
Seagrass		33.52	12.26	34.64	< 0.0001
	20				
Depth		4.61	2.49	180.83	< 0.0001
Coral		7.39	6.00	0.53	0.4681
Boulder		34.89	7.00	122.92	< 0.0001
Cobble		4.32	36.33	33.31	< 0.0001
Gravel		0.00	0.56	3.71	0.0574
Sand		18.07	24.11	1.85	0.1771
Seagrass		23.52	26.00	0.20	0.6576

TABLE 2. Comparisons of functional feeding groups (FFG) and taxa per group by distance from shore (m) between rocky and sandy beach habitats at St. John USVI, July 2007 and July 2008. Functional feeding group designations from Halpern and Floeter (2008).

Distance from shore (m)	Functional Feeding Group	FFG Richness		Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
1	Diurnal planktivore	2	1	Atherinidae	X	
				Engraullidae	X	X
	Macrocarnivore	1		Gerres cinereus	X	
	Piscivore	1		Eucinostomus jonesi	X	
	Territorial algae detritivore	1		Stegastes leucostictus	X	
	Group Total	5	1			
3 Diurnal planktivore	3	2	Atherinidae	X		
			Engraullidae	X	X	
			Thallasoma bifasciatum	X		
			Opistognathis macrognathus		X	
	Macrocarnivore	5	5	Dasyatus americana		X
				Gerres cinereus	X	X
				Lutjanus apodus	X	
				Lutjanus synagris	X	X
				Ocyurus chrysurus	X	X
				Sphyraena barracuda	X	X
	Mobile benthic invertivore	6	2	Haemulon flavolineatum	X	X
				Haemulon sciurus	X	
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	X
				Halichoeres maculipinna	X	
				Halichoeres radiatus	X	
	General omnivore	2	1	Abudefduf saxatilis	X	X
				Abudefduf taurus	X	
	Piscivore	4	1	Ablennes hians	X	X

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TABLE 2. Continued

Distance from shore (m)	Group	FFG R	ichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitat
				Carcharhinus perezi	X	
				Eucinostomus jonesi	X	
				Holocentrus rufus	X	
	Sand invertivore	1	0	Pseudupeneus maculatus	X	
	Scraper	4	4	Acanthurus bohianus	X	X
				Acanthurus chirurgus	X	
				Scarus taeniopterus	X	
				Sparisoma aurofrenatum	X	
				Scartella cristata		X
				Bathygobius soporator		X
				Synodus saurus		X
	Coral colonial sessile invertivore	2	1	Chaetodon capistratus	X	
	sessile invertivore			Chaetodon striatus		X
				Sphoeroides testudineus	X	
	Territorial algae detritivore	1	2	Stegastes diencaeus		X
				Stegastes leucostictus	X	X
	Turf grazer	1	1	Acanthurus coeruleus	X	X
	Group Total	29	19			
6	Diurnal planktivore	2	5	Atherinidae		X
				Engraullidae		X
				Harengula humerali		X
				Thallasoma bifasciatum	X	X
				Mugil curema	X	
				Opistognathis macrognathus		X
	Excavator eroder	1	0	Sparisoma viride	X	
	Macroalgae browser	2	0	Archosargus rhomboidalis	X	
				Sparisoma radians	X	
	Macrocarnivore	5	5	Dasyatus americana		X

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	ichness	Taxa	Occurrence	
(400mm) (500mm) (500mm)		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
				Gerres cinereus	X	X
				Lutjanus apodus	X	
				Lutjanus synagris	X	X
				Ocyurus chrysurus	X	X
				Sphyraena barracuda	X	X
	Mobile benthic	10	4	Trachinotus goodei	X	X
	invertivore			Haemulon aurolineatum	X	
				Haemulon flavolineatum	X	X
				Haemulon sciurus	X	
				Haemulon striatum	X	
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	X
				Halichoeres maculipinna	X	
				Halichoeres radiatus	X	
				Scarus iserti	X	X
	General omnivore	1	1	Abudefduf saxatilis	X	X
	Piscivore	3	2	Ablennes hians	X	X
				Eucinostomus jonesi	X	
				Holocentrus rufus	X	
				Caranyx ruber		X
	Sand invertivore	1	3	Pseudupeneus maculatus	X	X
				Bothus lunatus		X
				Bothus ocellatus		X
	Scraper	9	4	Acanthurus bohianus	X	X
				Acanthurus chirurgus	X	
				Scartella cristata	X	X
				Bathygobius soporator	X	X
				Gymnothorax funebris	X	

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	Lichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
				Holacanthus ciliaris	X	
				Scarus taeniopterus	X	
				Sparisoma aurofrenatum	X	
				Sparisoma rubripinne	X	
				Synodus saurus		X
	Coral colonial	3	2	Chaetodon capistratus	X	
	sessile invertivore			Chaetodon striatus	X	X
				Sphoeroides testudineus	X	X
	Territorial algae	2	3	Stegastes diencaeus	X	X
	detritivore			Stegastes leucostictus	X	X
				Stegastes variabilis		X
	Turf grazer	1	1	Acanthurus coeruleus	X	X
	Group Total	40	30			
9	Diurnal planktivore	3	4	Atherinidae		X
				Harengula humerali		X
				Cheilopogon melanurus	X	
				Thallasoma bifasciatum	X	X
				Mugil curema	X	X
	Excavator eroder	1	1	Sparisoma viride	X	X
	Macroalgae browser	2	0	Archosargus rhomboidalis	X	
				Sparisoma radians	X	
	Macrocarnivore	5	6	Dasyatus americana		X
				Gerres cinereus	X	X
				Lutjanus apodus	X	
				Lutjanus synagris	X	X
				Ocyurus chrysurus	X	X
				Sphyraena barracuda	X	X

TABLE 2. Continued

Distance from shore (m)	Group	FFG R	ichness	Taxa	Occurrence	
Calcinia minum Gillaria o Carrino II Alimona		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
				Urolophus jamaicensis		X
	Mobile benthic invertivore	11	6	Trachinotus goodei	X	X
				Haemulon aurolineatum	X	
				Haemulon flavolineatum	X	X
				Haemulon sciurus	X	X
				Haemulon striatum	X	
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	X
				Halichoeres maculipinna	X	X
				Halichoeres radiatus	X	
				Lachnolaimus maximus	X	
				Scarus iserti	X	X
	General omnivore	3	0	Abudefduf saxatilis	X	
				Abudefduf taurus	X	
				Diplodus argenteus	X	
	Piscivore	6	2	Ablennes hians	X	X
				Caranyx ruber		X
				Eucinostomus jonesi	X	
				Holocentrus rufus	X	
				Acanthostracion quadricornis	X	
				Nicholsina usta	X	
				Pareques acuminatus	X	
	Sand invertivore	1	2	Pseudupeneus maculatus	X	X
				Bothus ocellatus		X
	Scraper	7	4	Acanthurus bohianus	X	X
				Acanthurus chirurgus	X	X
				Gymnothorax funebris	X	
				Holacanthus ciliaris	X	
				Scarus taeniopterus	X	X

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	ichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitat
				Sparisoma aurofrenatum	X	
				Sparisoma rubripinne	X	X
	Coral colonial sessile invertivore	4	1	Chaetodon capistratus	X	
				Chaetodon striatus	X	
				Epinephelus striatus	X	
				Sphoeroides testudineus	X	X
	Territorial algae detritivore	2	1	Stegastes diencaeus	X	
	detritivore			Stegastes leucostictus	X	X
12 Diurnal planktivore	3	2	Harengula humerali		X	
			Cheilopogon melanurus	X		
			Thallasoma bifasciatum	X	X	
				Mugil curema	X	
	Macroalgae browser	2	0	Archosargus rhomboidalis	X	
				Sparisoma radians	X	
	Excavator eroder	1	1	Sparisoma viride	X	X
	Macrocarnivore	4	5	Dasyatus americana		X
				Gerres cinereus		X
				Lutjanus apodus	X	
				Lutjanus synagris	X	X
				Ocyurus chrysurus	X	X
				Sphyraena barracuda	X	X
	Mobile benthic invertivore	10	4	Trachinotus goodei	X	
	mvertivoie			Haemulon aurolineatum	X	
				Haemulon flavolineatum	X	
			Haemulon melanurum	X		
				Haemulon sciurus	X	
				Haemulon striatum	X	
				Anisotremus surinamensis		X

## VIRGIN ISLAND FISH ECOLOGY

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	ichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
				Halichoeres bivittatus		X
				Halichoeres maculipinna		X
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	
				Halichoeres maculipinna	X	
				Lachnolaimus maximus	X	
				Scarus iserti		X
	General omnivore	3	0	Abudefduf saxatilis	X	
				Abudefduf taurus	X	
				Diplodus argenteus	X	
	Piscivore	5	1	Ablennes hians	X	X
				Holocentrus rufus	X	
				$A can tho stracion\ quadricorn is$	X	
				Nicholsina usta	X	
				Pareques acuminatus	X	
	Sand invertivore	2	1	Pseudupeneus maculatus	X	X
				Calamus calamus	X	
	Scraper	7	3	Acanthurus bohianus	X	X
				Acanthurus chirurgus	X	
				Bathygobius soporator	X	
				Holacanthus ciliaris	X	
				Scarus taeniopterus	X	X
				Sparisoma aurofrenatum	X	
				Sparisoma rubripinne	X	X
	Coral colonial sessile invertivore	3	0	Chaetodon capistratus	X	
				Chaetodon striatus	X	
				Epinephelus striatus	X	
	Territorial algae detritivore	2	1	Stegastes diencaeus	X	
				Stegastes leucostictus	X	X

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	ichness	Taxa	Occur	rence
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
	Turf grazer	1	1	Acanthurus coeruleus	Х	X
	Group Total	43	19			
15	Diurnal planktivore	3	3	Engraullidae		X
				Harengula humerali		X
				Cheilopogon melanurus	X	
				Thallasoma bifasciatum	X	X
				Mugil curema	X	
	Macroalgae browser	2	0	Archosargus rhomboidalis	X	
				Sparisoma radians	X	
	Excavator eroder	0	1	Sparisoma viride		X
	Macrocarnivore	4	5	Dasyatus americana		X
				Gerres cinereus	X	X
				Lutjanus analis		X
				Lutjanus apodus	X	
				Lutjanus synagris	X	X
				Ocyurus chrysurus	X	X
	Mobile benthic	10	6	Anisotremus surinamensis		X
	invertivore			Trachinotus goodei	X	
				Haemulon aurolineatum	X	
				Haemulon flavolineatum	X	X
				Haemulon melanurum	X	
				Haemulon sciurus	X	X
				Haemulon striatum	X	
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	X
				Halichoeres maculipinna	X	X
				Lachnolaimus maximus	x	
				Scarus iserti		X

## VIRGIN ISLAND FISH ECOLOGY

TABLE 2. Continued

Distance from shore (m)	Group	FFG R	lichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
	General omnivore	3	2	Abudefduf saxatilis	Х	Х
				Abudefduf taurus	X	
				Diplodus argenteus	X	
				Lactophrys triqueter		X
	Piscivore	3	0	Ablennes hians	X	
				Holocentrus rufus	X	
				Pareques acuminatus	X	
	Sand invertivore	2	0	Pseudupeneus maculatus	X	
				Calamus calamus	X	
	Scraper	5	3	Acanthurus bohianus	X	X
				Acanthurus chirurgus	X	
				Holacanthus ciliaris	X	
				Scarus iserti	X	
				Scarus taeniopterus	X	X
				Sparisoma rubripinne		X
	Coral colonial sessile invertivore	2	0	Chaetodon striatus	X	
				Epinephelus striatus	X	
	Territorial algae detritivore	1	1	Stegastes leucostictus	X	X
	Turf grazer	1	1	Acanthurus coeruleus	X	X
	Group Total	36	22			
20	Diurnal planktivore	2	4	Atherinidae		X
				Engraullidae		X
				Harengula humerali		X
			Thallasoma bifasciatum		X	
			Cheilopogon melanurus	X		
				Mugil curema	X	
	Macroalgae browser	2	0	Archosargus rhomboidalis	X	
				Sparisoma radians	X	

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG R	ichness	Taxa	Occurrence	
		Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats
a	Excavator eroder	0	1	Sparisoma viride		X
	Macrocarnivore	3	4	Lutjanus apodus	X	X
				Lutjanus synagris		X
				Ocyurus chrysurus	X	X
				Sphyraena barracuda	X	
				Urolophus jamaicensis		X
	Mobile benthic	8	5	Trachinotus goodei	X	
	invertivore			Haemulon aurolineatum	X	
				Haemulon flavolineatum		X
				Haemulon melanurum	X	
				Haemulon parra		X
				Haemulon sciurus	X	x
				Haemulon striatum	X	
				Myripristis jacobus	X	
				Halichoeres bivittatus	X	X
				Lachnolaimus maximus	X	
				Scarus iserti		X
	General omnivore	2	1	Abudefduf saxatilis	X	X
				Abudefduf taurus	X	
	Piscivore	4	1	Ablennes hians	X	X
				Holocentrus rufus	X	
				Nicholsina usta	X	
				Pareques acuminatus	X	
	Sand invertivore	2	1	Pseudupeneus maculatus	X	X
		_	•	Calamus calamus	X	
	Scraper	6	3	Acanthurus bohianus	X	X
	£	-		Holacanthus ciliaris	X	-
				Scartella cristata		X
				Scarus iserti	X	

TABLE 2. Continued

Distance from shore (m)	Functional Feeding Group	FFG Richness		Taxa	Occurrence		
	A PANA PANA PANA PANA PANA PANA PANA PA	Rocky beach habitats	Sandy beach habitats		Rocky beach habitats	Sandy beach habitats	
				Scarus taeniopterus	X	X	
				Sparisoma aurofrenatum	X		
				Sparisoma rubripinne	X		
	Coral colonial sessile invertivore	2	0	Chaetodon striatus	X		
				Epinephelus striatus	X		
	Territorial algae detritivore	2	1	Stegastes diencaeus	X		
				Stegastes leucostictus	X	X	
	Turf grazer	2	1	Acanthurus coeruleus	X	X	
				Sparisoma rubripinne	X		
	Group Total	35	22				

TABLE 3. Average numbers of fish taxa observed in combined transects of nearshore rocky habitats at Francis, Great Lameshur, and Little Lameshur beaches of St. John USVI, July 2007 and July 2008. Underscored means do not differ significantly (p=0.05).

Distance from shore (m)	15	6	9	20	12	3	1
Avg. no. species	5.9	5.0	4.8	4.1	3.3	2.7	1.0
F=1.42; p=0.2025							

TABLE 4. Average numbers of fish taxa observed in combined transects at sandy habitats at Francis, Great Lameshur, and Little Lameshur beaches on St. John, USVI, July 2007 and July 2008. Underscored means do not differ significantly (p=0.05).

Distance from shore (m)	9	6	12	15	20	3	1
Avg. no. species	20.0	18.0	17.8	12.8	11.0	9.0	3.0
F=2.93; p=0.0254							

TABLE 5. Comparison of average number of species at nearshore rocky and sandy habitats at Francis, Great Lameshur and Little Lameshur beaches, St. John, USVI, July 2007 and July 2008.

	Average number of species							
Distance from Shore	Rocky habitat	Sandy habitat	F	p value				
1	3.00	1.00	99.99	<0.0001				
3	9.00	2.67	8.43	0.0104				
6	18.00	5.00	79.85	< 0.0001				
9	20.00	4.89	56.38	< 0.0001				
12	17.75	3.27	37.54	< 0.0001				
15 20	12.80 11.00	5.89 3.73	4.93 6.73	0.0464 0.0159				

TABLE 6. Frequency of occurrence of taxa per functional feeding group at combined nearshore rocky habitats at Francis, Great Lameshur, and Little Lameshur beaches at St. John USVI, July 2007 and July 2008.

		Ι	Distance	from s	hore (1	n)	
	1	3	6	9	12	15	20
Diurnal planktivores	3	6	6	6	4	4	2
Excavators/eroders	0	0	1	1	1	0	0
Macroalgae browsers	0	0	1	1	1	1	1
Macrocarnivores	1	10	17	15	8	9	3
Mobile benthic invertivores	0	12	31	33	21	17	12
General omnivores	0	5	5	7	4	8	5
Strict piscivores	1	4	7	11	7	5	5
Sand invertivores	0	3	4	4	2	2	2
Scrapers	0	4	19	20	11	9	7
Coral/colonial sessile insectivores	0	3	6	7	5	2	2
Territorial algae/detritivores	1	5	5	8	2	2	2
Turf grazers	0	2	6	6	4	4	2
Total	6	54	108	119	70	63	43

TABLE 7. Frequency of occurrence of taxa per functional feeding group from combined nearshore sandy habitats at Francis, Great Lameshur, and Little Lameshur

beaches at St. John USVI, July 2007 and July 2008.

	Distance from shore (m)						
Functional feeding group	1	3	6	9	12	15	20
Diurnal planktivores	2	3	8	7	4	5	5
Excavators/eroders	0	0	0	2	1	2	1
Macrocarnivores	0	7	12	17	8	8	6
Mobile benthic invertivores	0	8	11	15	9	14	11
General omnivores	0	1	1	0	0	3	2
Strict piscivores	0	1	8	4	1	0	1
Sand invertivores	0	0	7	7	4	4	3
Scrapers	0	4	6	9	6	9	6
Coral/colonial sessile insectivores	0	1	4	1	0	0	0
Territorial algae/detritivores	0	4	5	4	2	4	7
Turf grazers	0	3	4	4	1	4	3
Total	2	32	66	70	36	53	45

TABLE 8. Comparison of taxa richness, diversity (H), and evenness of functional feeding groups between rocky and sandy habitats at combined Little Lameshur, Francis Beach, and Great Lameshur, St. John USVI, July 2007 and July 2008.

			I	Distance fr	om shore (	(m)	
		3.00	6.00	9.00	12.00	15.00	20.00
Richness	Rocky	7.00	10.00	10.00	8.67	8.00	6.67
	Sandy	5.33	8.33	7.33	5.67	6.67	7.33
	t value	5.57	16.89	11.4	6.14	10.26	6.22
	p>t	0.0020	<.0001	<.0001	0.0017	0.0002	0.0016
H Index	Rocky	1.84	2.04	2.05	1.91	1.90	1.53
	Sandy	1.43	2.02	1.82	1.55	1.77	1.86
	t value	8.05	60.02	26.46	10.23	18.96	7.82
	p>t	0.0005	<.0001	<.0001	0.0002	<.0001	0.0005
Evenness	Rocky	0.9764	0.8896	0.8927	0.8939	0.9171	0.9061
	Sandy	0.9539	0.9551	0.9226	0.9309	0.9606	0.9425
	t value	60.03	53.53	89.59	67.62	62.84	77.93
	p>t	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

## A Habitat Model for the Detection of Two-lined Salamanders at C. F. Phelps Wildlife Management Area, Fauquier and Culpeper Counties, Virginia

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#### **ABSTRACT**

Aquatic salamanders represent an important component of Virginia river watersheds, but despite potential declines, few specifics are known about their habitat preferences. We surveyed the habitats of the northern two-lined salamander and collected data on an array of habitat variables associated with the species. We used a logistic regression analysis to develop a model predicting its presence or absence for a given 50m-transect. Our final model incorporated the variation in stream depth and direction of stream flow and accounted for 25% of the variation in our data. We conclude that stream depth variation is an important feature of salamander habitat ecology, and surmise that direction of flow is of site-specific importance possibly related to stream order. Both features may be behavioral adaptations to avoid fish predation.

#### INTRODUCTION

Stream-dwelling salamanders are an important component of aquatic ecosystems. They account for a significant proportion of the biomass of a stream ecosystem, and act as a key trophic link, important as both predators and prey (Spight 1967, Burton and Likens 1975, Rocco and Brooks 2000). Consequently, these salamanders have potential to act as an indicator of stream health (Rocco and Brooks 2000, Barr and Babbitt 2002). This is particularly true for headwater streams were salamanders may act as the dominant vertebrate predator (Davic and Welsh 2004). Accordingly, it would be beneficial to better understand how these species make use of their available habitat. This is especially important in the face of on-going amphibian declines (Alford and Richards 1999). Knowledge of this type may provide better insights into the conservation of these species and their associated ecosystems (Cushman 2005).

Previous surveys of stream and terrestrial amphibian diversity have been carried out in the Rappahannock River watershed of northern Virginia; however, more needs to be done to quantify the habitat preferences of important stream species (Mitchell 1998, McGhee and Killian 2010). To begin addressing this need, we conducted a preliminary study of salamander habitat at C.F. Phelps Wildlife Management Area (WMA) located in the Rappahannock River watershed and developed a simple habitat model for the

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northern two-lined salamander (*Eurycea bislineata*), a common stream species for the area (McGhee and Killian 2010).

Northern two-lined salamanders are common to northern Virginia forest streams within the Rappahannock River watershed (Mitchell and Reay 1999). While they are considered potentially important components of the local ecosystems in which they occur, few studies have developed predictive models of habitat use (Davic and Welsh 2004). They occupy stream margins and seeps, using submerged rocks and woody debris for cover; but may periodically be found in upland terrestrial sites (Petranka 1998). Females attach eggs beneath submerged rocks of varying surface area in headwater streams (Jakubanis et al. 2008). Larvae of this species are benthic predators associated with stream pools with low silt (Smith and Grossman 2003, Petranka 1998). Two-lined salamanders are able to access low-order streams typically inaccessible to predatory fishes, and have become adapted to these headwater stream environments (Vannote et al. 1980, Davic and Welsh 2004). We hypothesized that two-lined salamanders would be detected in or near cool narrow, shallow streams. From this hypothesis, we predicted that important habitat variables in a logistic regression model would be stream temperature, stream depth, and stream width.

#### **METHODS**

We chose sampling sites by randomly selecting a GPS starting location constrained to occur within C. F. Phelps WMA, and moving from that point to the nearest stream. We then moved upstream or downstream a randomly selected distance of up to 50m, and laid a 50m transect running downstream. We sampled stream transects by searching five 1-m² quadrats placed within each of the five 10-m sections of the transect. The particular location of the quadrat within these 10-m sections was randomly selected (Jaeger 1994, Jaeger and Inger 1994). We searched quadrats by looking under larger cover objects such as rocks or decaying logs, leaf pack, leaf litter, and using a standard-mesh aquarium dip net (1/16 inch mesh size) to sample stream bottoms (Mitchell 2000). We identified captured salamanders to species (Petranka 1998). Data were collected at both transect and quadrat levels (Table 1).

We used logistic regression to select models with those predictive variables most associated with salamander captures at the transect level. Variables measured at the quadrat level were averaged and averages and standard deviations were used as separate predictor variables. As synergistic effects may occur between the variables we measured, we created *a priori* multiplicative variables for testing as well (Table 1). We used forward stepwise selection (P = 0.05 to enter and 0.10 to remove) in SPSS (SPSS Inc., Chicago IL). We assessed variable coefficients using the change in -2 loglikelihood and evaluated the explanatory value of models using Nagelkerke's  $r^2$  (Ryan 1997, Hosmer and Lemeshow 1989, Nagelkerke 1991). For all statistical analyses  $\alpha = 0.05$ .

#### **RESULTS**

From 13 April 2007 – 21 April 2009, we sampled 78 stream transects with 390 stream quadrats. We located 256 two-lined salamanders, 203 of which were larval. Two-lined salamanders were detected in 45 of the 78 stream transects, for a 58% encounter rate. Logistic regression selected two predictor variables: the standard deviation of maximum stream depth (SDMD:  $-0.12 \pm 0.06$  SE, change in -2 log

Table 1. Habitat variables for stream and terrestrial transect sites at C. F. Phelps Wildlife Management Area, Virginia. For variables that had a standard deviation (SD) associated with them, the SD was included in the analysis as a separate predictor.

Transect-Level	Quadrat-Level
Season <sup>a</sup>	Mean Maximum Depth
Relative Humidity	Maximum Depth SD
Vapor Pressure Deficit	Mean Stream Width
Air Temperature (C)	Stream Width SD
Air Pressure	Mean Depth*Width
Weather <sup>b</sup>	Depth*Width SD
Bank Habitat <sup>c</sup>	Mean Water Temperature
Direction of Stream Flow	Water Temperature SD
Slope of Stream Flow	1

<sup>&</sup>lt;sup>a</sup> Spring: Mar 20/21, summer: June 20/21, fall: Sep 22/23, winter: Dec 21/22

likelihood = 5.331, df = 1, P = 0.021), and direction of stream flow (Direction: 0.10 ± 0.01 SE, change in -2 log likelihood = 4.301, df = 1, P = 0.038, Figure 1). The model explained 25% of the variation in data ( $r^2$  = 0.25). Probability of predicting the detection of a two-lined salamander within a stream transect was equal to

This model would correctly predict the presence of two-lined salamanders in 84% of cases in our study site, and correctly predict the absence in 48% of cases. The standard deviation and the average of the maximum stream depth were positively correlated r = 0.75, P << 0.0001), and so the majority of transects with low variability in depth also tended to be shallow. Two-lined salamanders tended to be found in streams flowing both south and west (logistic regression  $\beta = 0.10$ , P = 0.05). No other variables or combinations thereof produced models of significant predictive value.

#### DISCUSSION

Our model indicated that two-lined salamanders are sensitive to variation in stream depth. As those streams with high depth variation tended to be generally deeper, we interpret this as a preference for shallower sites in avoidance of fish predators (Sih et al. 1992). The majority of our captures were larval, and Barr and Babbitt (2002) found that larval two-lined salamanders occurred in negative association with brook trout (*Salvelinus fontinalis*), a fish predator. Average maximum depth also tended to be chosen by models if depth SD and direction of stream flow were removed, reinforcing the likely importance of depth. Variation in depth may provide refuges for predators to feed on larvae, or larvae and adults may simply tend to avoid deeper sites. No salamanders were found in our study site at depths greater than 20 cm.

<sup>&</sup>lt;sup>b</sup> Clear, partly cloudy, overcast, light rain, medium rain

<sup>&</sup>lt;sup>c</sup> Deciduous, coniferous, mixed deciduous/coniferous, open field/shrub

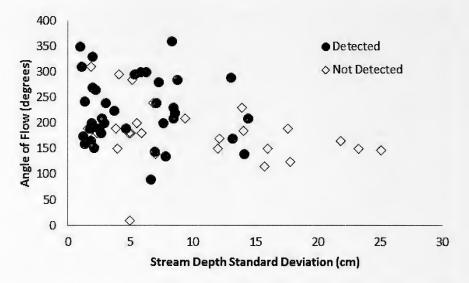


FIGURE 1. The relationship between angle of stream flow and stream depth variation for transects at C. F. Phelps Wildlife Management Area, Virginia. Salamanders were typically detected (circles) in streams with relatively low variability, flowing southwest.

The model's selection of stream flow direction as a predictor of the presence of two-lined salamanders is difficult to interpret. Individuals were most easily detected in streams flowing towards the south and west, towards the general direction of the bordering Rappahannock River. South and west flowing streams tended to flow either close to the Rappahannock or to be a 2<sup>nd</sup> or 3<sup>rd</sup> order stream, and larvae, which often drift downstream, may be attempting to find slow moving, shallow, or low depth-variance pools with sufficient cover (Petranka 1998, Barr and Babbitt 2002). Bruce (1986) found that first-year two-lined larvae tended to dominate downstream samples compared to upstream samples. Unfortunately, direction of stream flow is unlikely to translate this effect to other sites very well.

Interestingly, the model failed to include stream temperature. Grant et al. (2005) also failed to detect a water temperature effect for two-lined salamanders in the Shenandoah National Park, Virginia. Barr and Babbitt (2002) and Rocco and Brooks (2000), however, detected a positive relationship between two-lined salamander presence and temperature, but they may have found a greater range of temperatures concurrent with the greater elevation variability at their sites (300 - 1200 m) and 358 -752 m compared to our 200 - 400 m).

Our model was able to provide significant information on the habitat used by twolined salamanders using only two relatively easily acquired variables, and recommends itself for use as a preliminary predictor for presence/absence surveys when relatively few man-hours are available. It does tend to discount sites where the species does occur (false absences) about half the time, however, so more complete models are required to better understand the habitat ecology of the species.

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Project title: Central Mechanisms of Gastrin-Releasing Peptide-Induced

Anorexia in Chicks



### Andre Han, Virginia Tech

Faculty Advisor: Pablo Sobrano

Project title: Biomedical Characterization of Flavin Adenine Dinucleotide Dependent Monooxygenase from Aspergillus fumigates



Erin A. Haynes and Bowyn A. Wang

University of Mary Washington Faculty Advisor: Theresa Grana Project title: Nematodes in Virginia: Comparative Development and Model Organism Characteristics



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